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SOME RECENT RESULTS WITH CHEMICAL MUTAGENS

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Read to the Mendelian Society on September 29, 1950

ANYONE who in 1939 would have been asked to read a paper on chemical mutagenesis would have had an unenviable task. Unless he was prepared to go in detail over a mass of negative or inconclusive data, he would have found it difficult to spend even one hour on the subject. Today I am in the opposite position: so many substances have meanwhile been found capable of producing mutations that one is almost relieved to hear now and then of one which appears decidedly non-mutagenic. Apart from the mustards, formaldehyde is the only mutagen of which I have a thorough first-hand knowledge. I therefore shall confine myself to reviewing the results obtained with this compound, and to discussing a few general points relating to chemical mutagenesis.

RAPOPORT was the first to report a mutagenic effect of formalin on *Drosophila* (1946). His method consisted in mixing formalin with the food of the larvae in concentrations which allowed a proportion of them to develop into imagines. The emerging ♂♂ were subjected to a ClB test and about 6 % sex-linked lethals were obtained. This was an excellent result, corresponding to the effects of about 2000 roentgen units of X-radiation. KAPLAN in the United States repeated the experiment (1948), with a similar result.

My own work with formalin has been done mostly in collaboration with Dr. H. MOSER from Switzerland. I started out with a very definite problem in mind; but in pursuit of this main object a number of sidelines have opened up and have led to results which are interesting in their own right. My original problem was to find the actual mutagenic substance in the feeding tests with formalin. RAPOPORT takes it for granted that it is the formaldehyde itself which reacts with the proteins of the genes to produce mutations. This I found hard to believe. For-

This paper is the third and last of a series of talks on chemical mutagens, given in Lund in September, 1950. The first two lectures were given over to an analysis of the effects of mustard gas and related compounds.

malin is a very reactive substance. In the *Drosophila* experiments it is stirred into the warm food medium, which consists of agar, maizemeal, treacle and yeast, or some similar mixture. It seemed unlikely to me that free formaldehyde should not only fail to undergo reactions in the food medium, but should also pass through digestion and metabolism of the larvae, and still reach the chromosomes in the germ cells in its original form. It seemed more likely that some compound between formaldehyde and a food constituent was the actual mutagen.

I first attacked the problem from the negative side, trying to eliminate free formaldehyde as the responsible substance (AUERBACH, 1949). Adult ♂♂ and ♀♀ were exposed to sub-lethal doses of formaldehyde vapour and their progeny was tested for the occurrence of sex-linked lethals. With the possibility of a sensitive period during germ cell development in mind, I bred the treated animals for 4 periods of 5 days each, and tested samples of progeny from each of the four successive broods. Germ cells in successive broods must have been successively younger at the time of exposure. The result was uniformly negative. Exposure of larvae to vapour likewise failed to yield mutations. Quite recently negative results have also followed bathing of eggs with or without chorion for 1—2 hours in fairly high concentrations (2 % and 4 %) of formaldehyde in water. RAPOPORT (1947) claims that he obtained mutations by this kind of treatment. Unfortunately he does not describe his technique of treating and washing the eggs. In one of my series a very high mutation rate was in fact obtained (7 %); but this obviously was due to faulty technique. The eggs had been treated and washed on the agar slabs to which the flies had fastened them, and had then been transferred to food vials on tiny scraps of the agar medium. In spite of repeated rinsing with tap water, the agar seems to have contained sufficient formaldehyde to make the food mutagenic for the newly hatched larvae. Eggs of the same batch which had been treated and washed in exactly the same way, but had been transferred to food free from all traces of adhering agar medium did not develop mutations.

It certainly does not look as though formaldehyde by itself is a mutagen for *Drosophila*; but too much value should not be given to purely negative results in such experiments, because incidental conditions, such as penetration, toxicity and competitive reactions with other cell constituents may obscure the outcome. Dr. MOSER and I therefore started from the opposite, positive, angle and attempted to find the supposed mutagenic compound in treated *Drosophila* food.

First, however, we had to test another possibility. Formalin treated food is rather toxic. Larvae which are reared on it have a prolonged development, and the emerging flies are often small and underfed. It was known from older work that general starvation does not increase mutation rate in *Drosophila*. But it seemed conceivable that formaldehyde, by forming compounds with certain amino acids, might deprive the larvae of some essential food constituent, and that this specific deprivation might cause mutations. Feeding tests showed that this is not so. Starvation, far from enhancing the mutagenic effect of formalin, reduces it. When the food is enriched with either yeast or extra sugar, a given concentration of formaldehyde produces less severe disturbances of larval development, but more mutations. The reason for this negative correlation between toxicity and mutagenicity has not yet been studied. Selection may play a rôle. It is also conceivable that the mutagenic effect of formalin is influenced by speed or type of metabolism. Whatever the cause, this relationship between starvation and mutation rate shows that formalin produces mutations not negatively by deprivation, but positively by initiating some mutagenic reaction.

It therefore seemed possible that, if indeed a compound between formaldehyde and a food constituent should be the actual mutagenic agent, this would be effective when fed to the larvae over and above their ordinary diet. The food component most likely to react with formaldehyde are the amino acids of the proteins. We therefore treated casein with formaldehyde, washed it until a sensitive colour test (RIMINI and SHRYVER) failed to show more than traces of free formaldehyde in the wash water, and added the formalinised washed casein to the ordinary medium of the larvae. About 3 % sex-linked lethals were obtained, an undoubted positive result. However there was one weak point in this experiment. Many of the reactions between amino acids and formaldehyde are easily reversible. Such labile compounds might have given off free formaldehyde during the period of treatment, and the released formaldehyde rather than the original compound with protein might have been the effective mutagen.

We therefore repeated the experiment with the modification that washing of the formalinised casein was continued for so long, that two days in water of 25° C failed to set free more than traces of formaldehyde from the washed product. With this substance no mutations were obtained. It looks as though we may have to come back to a modified version of RAPOPORT's assumption. It seems possible that free formaldehyde is indeed the actual mutagen, but that the necessary, or

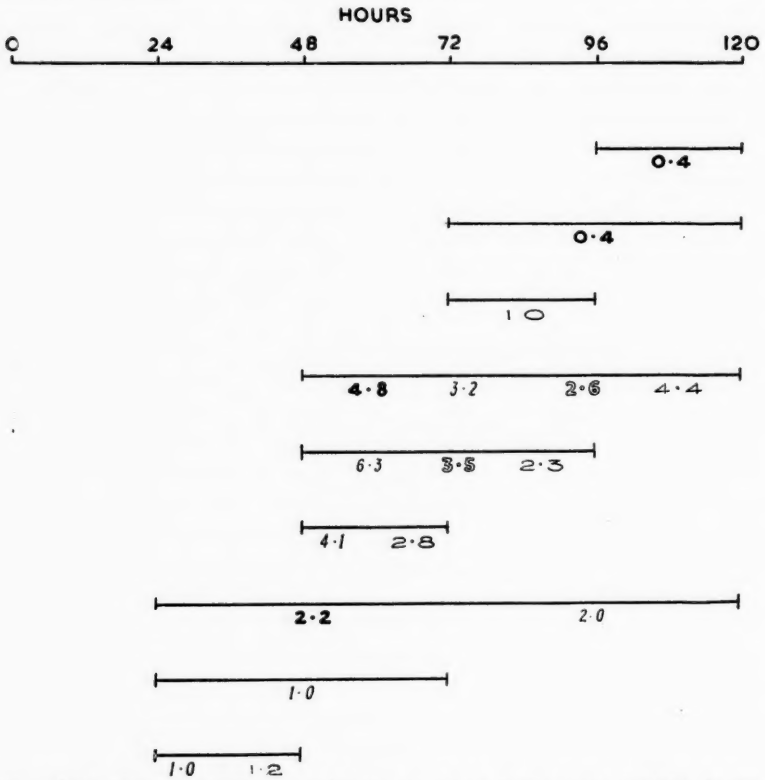

Fig. 1. Data from experiments in which the age of the larvae is the variable factor. (See text.)

at least the most favourable, condition for its effect is a slow release, in only slightly toxic doses, from labile compounds with protein either into the food medium, or into the digestive tract of the larvae, or perhaps even into the germ cells themselves.

As I said at the beginning of this lecture, this work has branched out into several sidelines. One of these started from the first experiment in which the feeding technique was used. In order to obtain sufficient progeny I had kept the treated ♂♂ for two periods of five days each. Mutation rate in the first brood was 6,3 % sex-linked lethals, in the second, 2,7 %. This suggested the existence of a sensitive period. In feeding experiments, two different types of sensitive period have to be considered. One involves the age at which the larvae are treated, the

other the stage of the germ cells in treated larvae of a given age. Experiments were carried out to test both possibilities. Fig. 1 presents data from experiments in which the age of the larvae was the variable factor. The horizontal line at the top represents larval life under normal conditions. About 24 hours after the egg has been laid, the larva hatches. The first moult takes place after about one day, the second after another day, and the third instar lasts 2—3 days and leads up to pupation. On treated food, the various instars may be more or less prolonged. Larvae were transferred to treated food at different ages and were either left to complete pupation there, or were retransferred to normal medium after a given period. The short horizontal lines represent the periods spent on treated food. The figures give the frequencies of sex-linked lethals in the first progeny of ♂♂ which had undergone the treatment. Figures printed in the same type refer to different series from the same experiment. Significant increases in mutation rate, that is frequencies of about 2 % or more were obtained with all those exposures — and with only those — which included the period from 48 to 72 hours, that is the second instar. The one exception (1 % in the last but one horizontal) tends to prove the rule; for here the larvae had suffered so much from the treatment that most of them had not reached the second instar after 48 or even 72 hours.

These data seemed clear proof of the existence of a sensitive period during the second larval instar, and this was our original interpretation. However, subsequent experiments have led to a complete revision of our ideas on this point. First of all, we detected the negative correlation between toxicity and mutagenicity already mentioned. This had a bearing on the apparent lack of sensitivity during the first larval instar; for such young larvae are much more susceptible to the toxic action of the treated medium than older larvae and show clear signs of starvation. It therefore seemed possible that malnutrition or poisoning had masked an existing sensitivity at this early age. This, indeed, was found to be true. When we re-tested first-instar larvae on a medium which had been enriched with extra yeast and sugar, starvation was much less severe, and the mutation rate became equal to that found in second-instar larvae. There remained the apparent lack of mutations in older larvae. This could not be explained by starvation; for these larvae stand the treatment very well indeed. Until recently we thought that for some unanalysed reason, perhaps connected with metabolic rate or accessibility of the germ cells, these older larvae were insensitive to the mutagenic action of formaldehyde. The true explanation was found in

a study of the second type of sensitive period, which relates to the stage of the germ cells within larvae of a given age.

As has been mentioned before, this whole line of work started with the observation that in our first feeding experiment about twice as many mutations were found in the first brood as in the second. This observation has since been confirmed in practically every experiment in which successive broods have been obtained from larvae treated during the first or second instar. Thus in one representative experiment, ♂♂ which had spent their whole larval life on formalin treated food were given fresh virgin ♀♀ soon after emergence and then again three times at intervals of 5 days. Altogether 4 broods were obtained, representing germ cells which at the time of treatment had been successively younger. The frequency of sex-linked lethals in the four broods was: 14,1 %; 2,2 %; 0,2 %; 0,2 %. The last two frequencies are those usually found in the controls. When the same kind of experiment was carried out with ♂♂ which had been treated only during the third larval instar, no mutations were obtained in the first brood; but mutations did turn up in the second. In the third and fourth brood mutation frequency had again fallen to that of the controls.

It thus appears that sensitivity is not restricted to one particular instar, but is found in larvae of every age. There is, however, one particular stage in the development of the male germ cells which alone — or preferentially — responds to the treatment. In young larvae this stage is the most advanced one present in the testis; in older larvae, the most advanced germ cells have already passed the sensitive stage, and younger cells have taken their place.

The evidence on the nature of the sensitive stage is still incomplete. Primary spermatocytes seem to appear very early in larval life, secondary ones considerably later; but this still requires checking. In any case, cytological evidence is not sufficient to decide the question, because we do not know the lag between feeding and effect on the germ cells. Genetically, the problem can be approached by looking for clusters of identical mutations in the progeny of individual treated ♂♂. The presence of such clusters shows that the original mutation has taken place in a spermatogonium which by division produced the cluster. Absence of clusters cannot, of course, be taken as evidence that no spermatogonial mutations have occurred; for we can, after all, test only small samples of all spermatozoa, and these may fail to include more than one mutation out of a cluster. RAPOPORT specifically mentions the presence of clusters of lethals in his material (1947); but he

does not explain how he made sure that several sex-linked lethals obtained from the same treated ♂ were, in fact, identical. Since sex-linked lethals cannot be obtained in live ♂♂, the usual cross-tests for allelomorphism cannot be applied to them, and accurate location on the chromosome is laborious and never quite conclusive. To overcome this difficulty we carried out two experiments in which lethals on the second chromosome were scored. These lethals can be kept in heterozygous condition in both sexes, and cross-tests for identity can be done with them. Many of the ♂♂ in these experiments had more than one lethal in their progeny, some as many as 8 or 9. But with one exception, in which two lethals from one ♂ were identical, each seeming cluster consisted in reality of a number of different single lethals. Although such data cannot be taken as proof that no spermatogonial mutations were produced, they certainly do not make this likely. Thus, cytological and genetical evidence point to a sensitive period rather late in spermatogenesis, perhaps early in meiosis.

Another observation on differential sensitivity was made at an early stage of our work. In an experiment designed to yield autosomal lethals we tested ♀♀ instead of ♂♂. To our surprise no mutations were produced. Since at this time we believed that only second-instar larvae react to formalin, we had adopted the technique of transferring larvae from normal to treated food at what we thought was the beginning of the sensitive stage, *i. e.* one day after hatching from the egg. The simplest explanation for the lack of mutations in the female germ cells was that female larvae have their sensitive stage during the first instar, which with our technique had received no treatment. However, when we repeated the test on ♀♀ which had spent their whole larval life on treated food, the result was equally negative. There still remained two possibilities to consider. One was that the sensitive stage of female larvae is late in larval life, perhaps shortly before pupation; in this case the food might have lost its mutagenic ability before the time the female larvae had become responsive to it. The other was that in female larvae the most advanced germ cells have already passed their sensitive period, and that younger sensitive cells had not been represented in the first progeny which had been tested by us. To examine these two possibilities, a series of 16 tests was carried out. Larvae were transferred from normal to treated food immediately after hatching from the egg and again on each of the three next days, *i. e.*, up to about one day before pupation. A comparison of mutation rates between these 4 treatments should show whether there exists a sensitive stage at any one

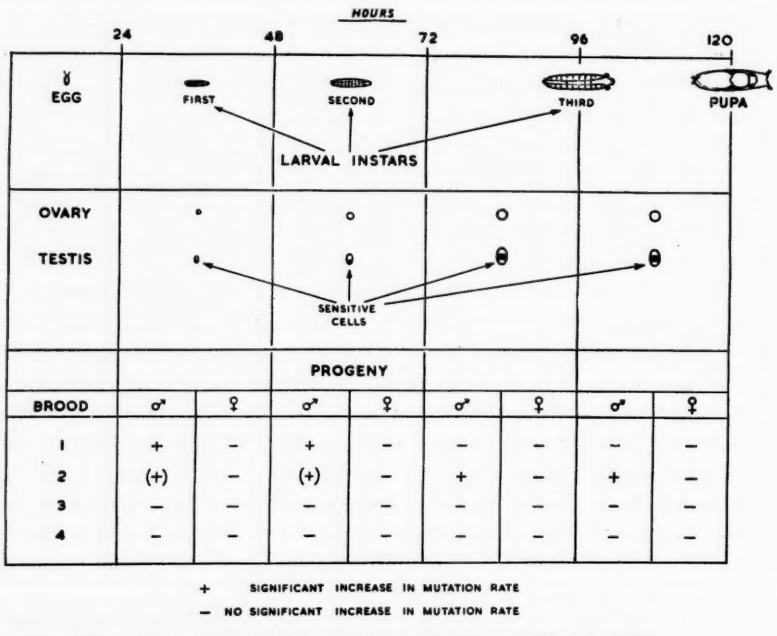

Fig. 2. Sensitivity of germ cells to the mutagenic action of formalin-treated food.

particular age of the female larva. The ♀♀ which emerged in these tests were made to produce progeny during 4 successive broods of 5 days each; a comparison of mutation rates between the 4 broods of each treatment should show whether at a given larval age there exists a particular developmental stage of the germ cell which responds to treatment. The result was uniformly negative. Thus female germ cells remain unaffected by formalin whatever the age of the larvae or the developmental stage of the germ cells within them. The position regarding sensitivity as it appears at present is summarized in Fig. 2. The top line again represents larval life, from the egg through three instars to pupation. The lower portion gives a rough summary of the data on which our conclusions are based. The four columns from left to right represent the four types of treatment: transfer of larvae to normal food at 24, 48, 72, and 96 hours after egg laying. The four rows represent the four successive broods — each produced during a 5-day period — during which progeny had been sampled and tested for lethals. The

+ and — signs in the 16 squares indicate success or failure in the mutation test. A (+) stands for a slight increase in mutation rate.

The middle part of the picture shows diagrammatically the position of the sensitive cells in larvae of different age and sex. In the larval testes they occupy the most advanced position during the first and second instar; during the third instar these cells have already passed their sensitive stage, and their place has been taken by a younger generation of cells. There are suggestions that it is the primary spermatocytes in which the sensitive period occurs. Larval ovaries, through the whole of development, do not appear to contain sensitive cells. Our original interpretation of this striking sex difference was that metabolism in ♀♀ differs from that in ♂♂ in such a way that the mutagenic compound either is not formed in sufficient quantity or is somehow prevented from reaching the germ cells. On the basis of what we now know about the sensitivity of male germ cells it seems possible that female germ cells never reach their sensitive period during larval life, and that a positive result might be obtained also with ♀♀ if it were possible to get the mutagen through feeding into the oocytes of young imagines. This is now being tested.

All these conclusions have been based on work with recessive lethals, but formalin, like mustard gas, is capable of producing all known types of mutation: dominant lethals, visible mutations — again as after mustard gas, unspecific in type —, small and large deletions, inversions, and translocations. Formalin resembles mustard gas and differs from X-rays in producing relatively few translocations as compared with lethals, and in often having a delayed effect. Indeed, the existence of a delayed effect is here even more easily established than for mustard gas. As after mustard gas treatment, about 50 % of all visible mutations appear first as mosaics, and gonadic mosaics for a lethal are frequent among apparent semi-lethals. Since formalin acts on young germ cells which have not yet undergone meiosis, the mere existence of these mosaics indicates a delay of mutation by more than one cell division.

The results with formalin have provided a striking illustration of the rôle which differences in sensitivity may play even between cells of the same organism. Obviously we cannot expect that a substance which produces mutations in one organism will always do so in all others. Very drastic and penetrating substances like the mustards may be exceptions; it is probable that mustards will produce mutations in every organism which is exposed to them. But for less effective sub-

stances discrepancies have already been found. Thus diazomethane is a good mutagen for *Neurospora* (JENSEN, KÖLMARK and WESTERGAARD, 1949), but not for *Drosophila* (JENSEN, WESTERGAARD and AUERBACH, unpublished). Conversely urethane produces translocations in flowering plants (OEHLKERS, 1943), and mutations in *Drosophila* (VOGT, 1948), but so far has failed to produce back mutations in *Neurospora* (WESTERGAARD, unpublished). Fortunately such divergences do not invalidate positive results; but they emphasize the need for caution in the interpretation of negative results which by a change of technique may become positive. An example is furnished by the attempts to produce mutations with formalin in *Neurospora*. American workers (DICKEY, CLELAND and LOTZ, 1949) reported that formalin by itself is not mutagenic for *Neurospora*, but that it acquires mutagenic ability when mixed with hydrogen peroxide, which in their experiments was not mutagenic when used by itself. WESTERGAARD and I have just repeated these experiments and have confirmed that a mixture of formaldehyde and hydrogen peroxide in aqueous solution is an excellent mutagen for *Neurospora*; but in addition, by varying the methods of treatment, we found that under suitable experimental conditions an aqueous solution of formaldehyde alone can have a very pronounced mutagenic action. There has not yet been time to analyse the necessary conditions in detail; but it is already clear that concentration and time of exposure play a rôle, long exposures to low concentrations being more effective than short ones to high concentrations. Other factors, such as developmental stage of the fungus and addition of nutrients to the formalin solution may also have an influence.

So far every chemical mutagen, which has been sufficiently analysed, has shown itself capable of producing chromosome breaks. Perhaps it is only to be expected that a substance which can react unspecifically with genes to produce mutations can also react with the chromosome thread to destroy its coherence. It may well be that the search for a substance which produces mutations but no chromosome breaks will turn out to be identical with the search for a specific mutagen which reacts with only one or a few genes. It is, however, unlikely that all chemical mutagens will produce chromosome breaks, re-arrangements and gene mutations in the same relative frequencies. Quantitative differences such as exist between different types of mutagenic radiation are to be expected. The question whether all chemically produced lethals in *Drosophila* contain the same percentage of small deficiencies, as do those produced by X-rays and by mustard gas (SLIZYNSKI and

SLIZYNSKA, 1946), is particularly interesting. Unfortunately, this type of work is very laborious and requires a specially trained cytologist; but its results would be rewarding and would help towards a better understanding not only of the action of chemical mutagens, but also of the nature of mutation in general.

Whilst it seems possible that a substance which produces mutations will in general be able to produce chromosome breaks, the reverse seems more doubtful. A great number of substances have been found capable of breaking the chromosomes, especially by LEVAN and his co-workers (LEVAN and TJIO, 1948). Only a few of these chromosome breaking compounds have as yet been tested for mutagenic ability. Pyrogallol, the most effective substance in the experiments of LEVAN and TJIO, failed to produce lethals in *Drosophila*. No great importance should be attributed to this negative result; nevertheless, it did not surprise me. In the tests with *Allium* roots, chromosome breaks caused by pyrogallol and other effective substances were rarely followed by reunion of broken chromosome ends; this type of chromosome fragmentation would not be expected to result in mutation. We must consider, however, that the *Allium* test is carried out on mitotic chromosomes. It cannot be taken for granted that meiotic chromosomes would always react in the same way. It would be very interesting if some of these chromosome-breaking substances could be tested on meiotic plant chromosomes. The effects produced on these would be a better guide to compounds worth testing for mutagenic capacity.

The ability of chemical mutagens to produce chromosome breaks as well as mutations forms part of their similarity with ionizing radiations. This similarity is very impressive indeed. So far, all sufficiently analysed chemical mutagens — the mustards, urethane, formalin — have been found capable of producing all the main effects caused by X-rays: lethals, visible mutations, small and large re-arrangements. We should not forget, however, that part of the similarity is due to our experimental methods. We design experiments to test for, say, lethals or translocations, and naturally what we obtain are lethals and translocations. Other effects may remain undetected because our methods are not suitable for uncovering them. Thus, in a ClB test with a nitrogen mustard an unusual type of segregation occurred in an F_2 culture (AUERBACH, 1947). Analysis showed that the centromere of the X-chromosome had been damaged in such a way that the chromosome tended to follow its homologue along the spindle, with the result that it either became enclosed in the sister cell or was lost on the spindle. Now F_2

cultures in a ClB test are usually examined quite superficially with the sole purpose of seeing whether or not they contain males. Often the examination is left to a laboratory assistant without genetical training. It was therefore pure chance that this unexpected type of effect was discovered. Other peculiar effects may have escaped our routine methods.

But even allowing for this limitation in our methods, the fact remains that all genetical effects which are produced by ionizing radiations can also be obtained by treatment with chemical mutagens. Some workers have taken this as evidence that ionizing radiations and chemical mutagens act in the same way. I do not find this argument convincing. After all, chromosomes and genes have limited ways of responding to a stimulus. A chromosome can break and a gene mutate. The formation of special types of re-arrangement, such as inversions and translocations, is a secondary process which probably takes place whenever free rejoinable chromosome ends have been produced by whatever kind of primary mechanism. In the same way as a muscle contracts in response to a variety of stimuli, it seems likely that different physical or chemical stimuli may cause a chromosome to break and a gene to mutate. At the moment, there seems to exist a general endeavour to find a theory of mutagenic action which would reduce mutagenesis by whatever physical or chemical agencies to one ultimate mechanism. It would, indeed, be most satisfactory if such a unifying theory could be proposed and proved. I only want to point out that in my opinion the known facts do not force us to assume that there is such a common mutagenic mechanism, except in the widest sense, that of energy transfer to points on the chromosomes, or chemical reactions with a constituent of the chromosome.

At the present state of knowledge it seems more profitable to restrict theories of mutagenic action to groups of related substances. One such group is formed by hydrogen peroxide, organic peroxides, and the mixture of hydrogen and formaldehyde which I mentioned before. If, as seems likely, radiation should act by means of activated atoms or radicals produced through the disintegration of water, this group of chemical mutagens would form a link with physical mutagenesis.

Another group of related mutagens is formed by vesicants of the mustard gas type, and lachrymators. Among the latter, mustard oil, allyl *iso*thiocyanate, is a decided, though weak mutagen. Chloracetone and dichloracetone gave doubtful results and probably are weak muta-

gens (AUERBACH and ROBSON, 1947). Various theories have been put forward to explain the mutagenic ability of these compounds. One of them considers that mutations are produced by action on —SH groups in the proteins of the chromosomes or in enzymes concerned with chromosome growth and reproduction (BACQ, 1946). Lachrymators act specifically on —SH groups. They inactivate —SH enzymes, in particular phosphokinases, which are concerned with phosphate transfer. The mustards, too, act on —SH groups, but this is only a minor part of their reaction pattern. They do, however, inactivate phosphokinases, although not by action on the —SH groups of these enzymes. The second theory considers the possibility that poisoning of phosphate transferring enzymes may be the primary step in the production of mutations (NEEDHAM, 1948). Since lachrymators as well as mustards inactivate these enzymes, although by a different mechanism, the first and second theory merge at this point. They were tested by exposing *Drosophila* to two known potent —SH poisons. One of them, lewisite, had been found negative in earlier work (AUERBACH and ROBSON, 1947). More recently chloropicrin has been tried. With the possibility of a sensitive period in mind, I kept the treated ♂♂ for about a fortnight, mating them to fresh virgin ♀♀ every 3—4 days. The result was uniformly negative, although progeny produced during the third period came from germ cells which show a maximum of sensitivity to the mutagenic action of mustard gas (AUERBACH, 1950). Thus the results with these two substances do not support either of the two theories; but, being negative, they cannot be used to invalidate these theories.

A third theory on the mutagenic action of the mustards is based on their chemical structure. All mustards are characterized by the presence of chloroethyl chains which are attached to a central sulphur or nitrogen atom. It is these chloroethyl groups which are the chemically active parts of the molecule. Early in the war it was noticed that mustards with only one chloroethyl group are disproportionately less toxic than those with two (OGSTON, 1948). Recently, workers at the Chester Beatty Institute, London, have carried out a study of the cytological effects of a number of substances of the mustard gas type. They found likewise that all their efficient substances had two chloroethyl chains (LOVELESS and REVELL, 1949). This led them to postulate that these compounds produce chromosome breakage and mutation by forming cross-links between neighbouring fibres of the chromosome. However, cross-linkage can hardly be a necessary condition for the production of mutations. For three groups of scientists, namely STEVENS

and MYLROIE in the United States, JENSEN, KIRK and WESTERGAARD in Denmark, AUERBACH and MOSER in Scotland (in press), have independently obtained mutations in *Neurospora* and *Drosophila* with mustards which have only one chloroethyl chain. However, the idea of cross-linkage has proved valuable as a working hypothesis; for in the search for other substances which might produce cross-linkage, workers at the Chester Beatty Institute have discovered that diepoxides are very similar to the mustards in their cytological effects (LOVELESS and REVELL, 1949) and induce a high mutation rate (BIRD, in press). Yet even for these substances, cross-linkage does not appear to be the essential factor; for experiments with *Drosophila* first carried out by RAPOPORT in Russia (1948) and subsequently by BIRD (in press) have shown that the same high mutagenic efficiency characterizes ethylene oxide and ethylene imine, neither of which are likely to be efficient in causing cross-linkage between protein chains.

Apart from attacking proteins, mustard gas reacts with nucleic acids. HERRIOTT (1948) found that viruses containing desoxyribonucleic acid are inactivated faster by mustard gas than those containing ribose nucleic acid, and that an unusually high sensitivity to inactivation by mustard gas is exhibited by the pneumococcus transforming principle, which presumably consists of pure desoxyribonucleic acid. These findings suggest that also in the production of genetical effects by mustard gas reactions with nucleic acids may play an important rôle; but inferences from viruses or transforming principle to genes, and from inactivation to mutation are so hazardous that this idea cannot be considered as more than a hypothesis.

The possibility that there may be peculiarities of effect which are common to all chemical mutagens remains to be dealt with. The three properties which, as I showed in the first two lectures, distinguish mustard gas from X-rays — a sensitive period prior to the formation of mature sperm, shortage of translocations, and delayed effect — are also characteristic of formalin. Analysis of other mutagens from this point of view has hardly been started; but there are already some data which show similar peculiarities for a few of them.

There are also certain similarities between chemically induced and so-called spontaneous mutations. Translocations are rare among spontaneous mutations as after chemical treatment. A delayed effect cannot, of course, be detected for spontaneous mutations, because we do not know the time when they occur; but the various unstable mutations which have been found in untreated material (DEMEREC, 1928) recall

the unstable condition which precedes a chemically induced delayed mutation. In *Drosophila*, spontaneous mutations are more frequent in ♂♂ than in ♀♀ (AUERBACH, 1941, 1949), and those occurring in ♂♂ are found preferentially in early broods (MULLER, 1945; LAMY, 1947): a striking parallelism with formalin induced mutations. In flowering plants LEWIS (1948) found a sudden increase of spontaneous mutation rate during meiotic prophase. Apparently there exist sensitive periods to whatever causes produce spontaneous mutations. It would be most interesting to find out whether the sensitive periods to natural causes and chemical mutagens coincide. If this should be true, it would fit in with the assumption that chemical mutagens which arise in metabolism or are introduced into the organism with its food may have played a rôle in evolution. It is interesting to note in this connection that mutations have, indeed, been produced in the laboratory with substances which occur in nature. Allyl *iso*thiocyanate occurs as a glycoside in *Brassica* plants. Even more suggestive is the finding by MARQUARDT (1949) that translocations in flowering plants can be produced by means of putrescin, a product of natural decay, or of extracts from old seeds. It would indeed be a great achievement if the work on chemical mutagens could contribute to our understanding of the origin of mutations in nature.

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CYTO-GENETIC PROPERTIES AND PRACTICAL VALUE OF TETRAPLOID RYE

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I. MATERIAL.

THE first autotetraploid plants of rye (*Secale cereale*) were produced by DORSEY (1936), using the heat shock method elaborated by RANDOLPH (1932). In 1936 the first autotetraploid barley strain was also produced with the heat chock method (MÜNTZING, TOMETORP and MUNDT-PETERSEN, 1936—37). Seeds of the tetraploid American rye and the tetraploid Swedish barley were exchanged and, thus, in the autumn of 1937 20 seeds of this tetraploid rye, derived from the variety »Rosen-rye», could be sown at Svalöf. This resulted in 13 mature plants, which yielded a total of about 700 seeds. Most of these seeds were used for sowing an isolation group. In the following years this strain was kept pure by isolation, and efforts were made to improve the practical value by selection. During this work the cyto-genetic properties and the yield were tested and compared with those of other tetraploid strains. In the following this tetraploid strain of »Rosen-rye» is referred to as the »American tetraploid».

Under Swedish environmental conditions the American tetraploid was found to have rather disappointing properties, being highly sterile and rather low-yielding. Thus, from a practical point of view the work with tetraploid rye did not seem to be promising. However, by and by new tetraploids of Swedish origin were produced and studied, and it was realized that the American tetraploid was much below the average of other tetraploid strains, and that these strains also differed *inter se* with regard to yield and other properties.

The next tetraploid strain of rye was derived from a spontaneous diploid-tetraploid chimaerical plant of the Swedish land variety »Östgöta gråråg». This chimaera has already been described in detail (MÜNTZING and PRAKKEN, 1941, pp. 294—297). A few seeds from the tetraploid spikes were obtained after pollination with the American tetraploid and also the reciprocal cross could be undertaken. By and by

the hybrid progeny was increased and developed into a special tetraploid strain denoted »Swedish × American».

After the discovery of the colchicine method a couple of new tetraploid strains of purely Swedish origin were produced. Thus, following colchicine treatment in the autumn of 1940 the first tetraploid plants of the varieties »Stålråg» (Steel-rye) and »Wasa II» were obtained in 1941. More recently tetraploid strains of the varieties »Sangaste», »Toivo» and »Kungsråg» (King-rye) have been developed. It should be observed that most of these strains are based on a rather low number of original individuals. Thus, tetraploid Steel-rye is derived from 9 plants and tetraploid Wasa II from only 3 individuals. Special efforts are now being made to introduce the genes of a fairly large number of diploids into the new tetraploid strain of King-rye, diploid King-rye being the most wide-spread and valuable of the present Swedish rye varieties.

Besides the primary tetraploids mentioned more material was obtained by making crosses between some of the primary strains. The hybrid progeny of the cross Östgöta gråråg × American tetraploid has already been mentioned. Other special hybrid strains were raised from the crosses tetraploid Steel-rye × tetraploid American and tetraploid Steel-rye × tetraploid Wasa II.

Of more general importance is a crossing-group started in the autumn of 1944 by mixing 1000 kernels of each of the four tetraploid rye strains available at that time, *viz.* »American», »Swedish × American», »Steel-rye» and »Wasa II». The seed mixture was sown in an isolated plot, allowing spontaneous intercrossing between the four components in the summer of 1945. In the following year about 75 per cent of the plants in the crossing-group must have been F_1-hybrids. In 1947 the crossing-group must have been composed of a mixture of individuals, representing double crosses ($F_1 \times F_1$), F_2, $F_1 \times$ P-strains and pure P-strains. In 1948 and 1949 the intercrossing proceeded still further, and the material in the crossing-group must now be rather well intercrossed and on an average homogeneous. Selection of the best individuals was undertaken from 1946 and may have favoured the products of intercrossing. At any rate, this crossing-group contains a larger number of genes and is based on a larger number of original diploid individuals than the »pure» tetraploid strains.

II. CYTOLOGY.

In the main tetraploid rye shows the cytology characteristic of other autotetraploids, *viz.* presence of quadrivalents at meiosis and

formation of a certain proportion of aneuploids in the offspring. The mode of meiosis has already been described in detail for the chimaerical plant of Östgöta gråråg (MÜNTZING and PRAKKEN, 1941). In a tetraploid spike of this plant there occurred an average number of 2,33 ring or chain quadrivalents, besides trivalents, bivalents and univalents. At first anaphase some univalents were observed to divide, giving rise to lagging at second anaphase and presence of eliminated chromosomes in the tetrads.

Attempts were made to study meiosis in the various tetraploid strains by fixing spikes in chrome-acetic-formalin or other fixatives, imbedding in paraffin and staining the sections with crystal violet. However, in this way it was very difficult to get useful slides, tetraploid rye apparently containing an excess of nucleic acid at meiosis, causing a certain degree of stickiness. Better results were obtained with permanent aceto-carmin squashes prepared according to the method described by LIMA-DE-FARIA (1948). By the pressure applied some metaphase groups were sufficiently spread to allow accurate counts of the number and types of chromosome configurations present. For valuable help with the preparation of these slides I am much indebted to Dr. LIMA-DE-FARIA. Counts of the somatic chromosome numbers were made in the usual way, the root tips being fixed in chrome-acetic-formalin and the sections stained with crystal violet. Before fixation the pots containing the seedlings were placed over night at a low temperature (about the freezing point) to induce a shortening of the chromosomes, facilitating the counts. The data gathered may be given under the following two headings.

1. MEIOSIS.

In the summer of 1949 a number of spikes from well-developed plants of four tetraploid strains were fixed in acetic-alcohol. The strains used for fixation were Steel-rye, Sangaste, Toivo and Crossing-group. Meiosis was studied in detail in one plant of each of these categories. Additional information was obtained from some other plants, and, finally, tetrads were examined from all plants available.

In the four plants especially studied, and found to have $2n = 28$, the frequency of multivalents (quadrivalents + trivalents) ranged from 0 to 7 (Table 1), the most frequent number being 4. According to the table, the average values of the four plants range from 3,95 to 4,29, but the differences between these values are not significant. The great majority of the multivalents are represented by quadrivalents. The

TABLE 1. *Frequency of multivalents at first metaphase in tetraploid rye.*

Variety	Number of quadrivalents (or trivalents + univalents) 0	1	2	3	4	5	6	7	No. of p. m. c. examined	M	Number per cell of quadrivalents	trivalents	univalents
Steel-rye		3	7	13	14	14	2	5	58	3,95	3,81	0,14	0,28
Toivo			2	8	14	7	5	2	38	4,29	4,05	0,24	0,34
Sangaste			4	6	10	7	3		30	3,97	3,80	0,17	0,23
Crossing-group	1	3	1	4	8	8	5		30	3,97	3,87	0,10	0,23

average number of trivalents was found to range from 0,10 to 0,24 (Table 1). By subtraction of these values from the average number of multivalents the average numbers of quadrivalents were obtained and found to range from 3,80 to 4,05. As only a minority of the cells studied could be used for counts it is possible that the average numbers of multivalents are in reality somewhat higher, cells with many multivalents generally being more difficult to analyse than cells with few multivalents.

In cells with trivalents a corresponding number of univalents were present, but in some cases non-conjunction occurred, one bivalent being replaced by two univalents. Hence, the average numbers of univalents were somewhat higher than those of the trivalents, ranging from 0,23 to 0,34 per cell (Table 1).

The most frequent types of quadrivalents were rings or chains in zig-zag orientation, but other types were also observed. The slides were not good enough to allow accurate counts of the different types. In the chimaerical plant of »Östgöta gråråg» zig-zag arranged rings and chains were most frequent (MÜNTZING and PRAKKEN, 1941, p. 296).

As illustrations to the data discussed above four complete complements at *first metaphase* or pro-metaphase are represented by Figs. 1—4. The complements are reproduced with the chromosomes in »natural» position, this position, however, being more or less disturbed by the pressure used when making the slides. Fig. 5 shows a separate quinquevalent composed of five chromosomes in zig-zag orientation. This multivalent was observed in a plant of Steel-rye having 29 chromosomes.

The chromosome distribution at *first anaphase* was also observed in the four plants used for the study of first metaphase. The results are summarized in Table 2.

In all four plants the most frequent distribution is 14—14. However, the percentages of cells showing this regular distribution ranged

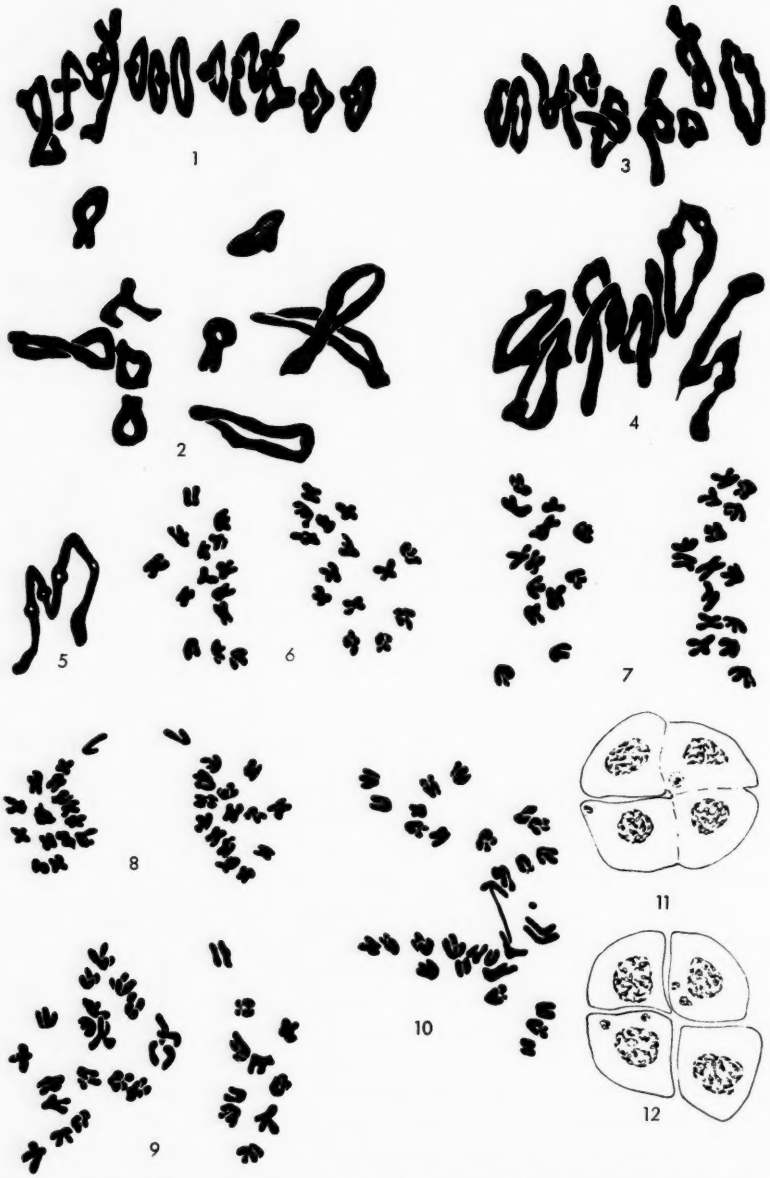
TABLE 2. *Chromosome distribution at I-A.*

Distribution	Tetraploid varieties:			
	Steel-rye	Sangaste	Toivo	Crossing-group
14 — 14	16	34	24	39
13 — 15	9	5	5	7
12 — 16	1			
13 — 1 — 14	3	1	13	5
13 — 2/2 — 14	4			1
12 — 4/2 — 14	1			1
13 — 2 — 13	1		1	
13 — 4/2 — 13	1			
12 — 1/2 — (15 + 1/2)	1			
12 — 2/2 — 15	1			
(14 + 2/2) — 2/2 — 12	1			
(12 + 2/2) — 6/2 — 12	1			
(14 + 1/2) — 1/2 — 13		2		
14 — 1/2 — (13 + 1/2)		1		
(13 + 1/2) — 3/2 — 13		1		
12 — 6/2 — 13				1
(13 + 1/2) — (14 + 1/2)			1	
Total	40	44	44	54
Per cent cells with 14 — 14 distribution	40,0	77,3	54,5	72,2
Per cent cells with divided or lagging univalents	35,0	11,4	34,1	14,8

from 40,0 in Steel-rye to 77,3 in Sangaste. More precisely the following ratios were obtained:

	14—14	Other distributions
Steel-rye	16	24
Sangaste	34	10
Toivo	24	20
Crossing-group	39	15

The heterogeneity of these ratios was examined with a χ^2-test and found to be significant, χ^2 being 16,02 and P intermediate between 0,01 and 0,001. Thus, there were significant differences between the slides examined with regard to the degree of regularity at I-A. Other data to be discussed below indicate that these differences are not typical of the four different strains but only of the individuals or even the flowers studied.



Figs. 1—12. Meiosis in autotetraploid rye. Figs. 1—4, first metaphase configurations in plants with $2n = 28$. Fig. 1, $3_{IV} + 8_{II}$; Fig. 2, $4_{IV} + 6_{II}$; Fig. 3, $6_{IV} + 2_{II}$; Fig. 4, 7_{IV}; Fig. 5, a separate quinquevalent from a plant with $2n = 29$. Figs. 6—10, first anaphase. Fig. 6, distribution 14—14; Fig. 7, distribution 13—15; Fig. 8, distribution 13—2/2—14; Fig. 9, distribution 9—6/2—17; Fig. 10, inversion bridge and fragment. Fig. 11, tetrad with 2 micronuclei; Fig. 12, tetrad with 4 micronuclei.

Next to 14—14 (Fig. 6) the distributions 13—15 (Fig. 7) and 13—1—14 were most frequent. In the latter case one chromosome is lagging between the anaphase groups and will in most cases divide later on. In cells in which this division had already occurred (Fig. 8) the distribution is denoted as 13—2/2—14. In the same way 12—4/2—14 in Table 2 means that 2 lagging chromosomes have divided. In some cells the half univalents had already reached one or both of the anaphase groups. Thus, for instance, in the cell showing the distribution 12 + 2/2—6/2—12 one anaphase group contains 12 normal chromosomes + a univalent represented by two separate chromatids, 6 chromatids from 3 divided univalents are still between the poles, and in the other anaphase group there are 12 undivided chromosomes. According to Table 2, a total of 17 different distributions were observed in the 182 cells examined. Already from this it is clear that a rather large proportion of aneuploid microspores must be formed.

As mentioned above, one of the plants of tetraploid Steel-rye had $2n = 29$. Chromosome distribution at first anaphase of this plant was studied, the following configurations and frequencies being observed:

14—15:	9 cells	12—6/2—14:	2 cells
13—2/2—15:	4 »	12—3—14:	1 cell
13—16:	4 »	11—4/2—16:	1 »
14—1—14:	3 »	11—1—17:	1 »
14—2/2—14:	3 »	12—1 + 4/2—14:	1 »
15—1—13:	1 cell	9—6/2—17:	1 »
12—2/2—16:	2 cells		

From these distributions it is rather certain that only a minority of the microspores will receive the euploid number 14, and that the proportion of such microspores will be smaller than in the euploid plants, having $2n = 28$.

Two cells at I-A of this plant are represented by Figs. 9—10. Fig. 9 represents the very unequal distribution 9—17 with 3 additional chromosomes dividing and lagging. Fig. 10, finally, shows the presence of a typical inversion bridge and fragment.

Interphase was highly regular, showing that in most cases the half univalents are included in the daughter nuclei. Only in a few of the cells one or two micronuclei were present. The percentages of cells with micronuclei were found to be 7,9; 1,0; 1,0 and 8,1 in the four plants representing the tetraploid varieties Steel-rye, Crossing-group, Sangaste

and Toivo. These values are based on counts of between 100 and 200 cells.

At *second anaphase* a good deal of lagging was observed as must be expected from the rather high proportion of division at I-A. In the plant of the crossing-group this stage did not occur. In the other plants examined the following results were obtained:

Tetraploid variety	Number of laggards				Per cent cells with laggards
	0	1	2	3	
Steel-rye, Plant 1	73	18	8	1	27,0
» » 2	72	17	9	1	27,3
Sangaste, » 1	99	6	1		6,6
Toivo, » 1	70	22	8		30,0

Steel-rye, Plant 2, is a plant in which the previous stages could not be studied in detail. Some I-A groups were seen, however, showing that also this plant had $2n = 28$. The other three plants in the above table are the same as those giving the data in Table 2. At first anaphase the plant of Sangaste was remarkably regular, and this is reflected by a low frequency of laggards at second anaphase, the percentage value being as low as 6,6. In Steel-rye and Toivo the percentage of cells with dividing or lagging univalents at I-A was higher, and this is evidently also true of the chromosomes lagging at II-A.

In the *tetrads* the frequency of micronuclei or microcytes is a rather good measure of the degree of meiotic irregularity. Hence, counts of this kind were undertaken not only in the plants especially studied with regard to meiosis but in all the other tetraploid plants available. Thus, data were obtained from 8 plants of tetraploid Steel-rye, 6 plants of the crossing-group, 8 plants of tetraploid Sangaste and 12 plants of tetraploid Toivo. From each plant at least 100 tetrads were examined. In most cases the eliminated chromosomes were present as micronuclei in the microspores (Figs. 11—12), but sometimes a cell wall had been formed leading to the formation of a special microcyte in addition to the four normal microspores. During the counts these two categories were not separated. As a rule, the number of micronuclei (including microcytes) ranged from 1 to 4 and only in rare cases higher numbers were observed (up to 7). For each plant the average number of micronuclei per tetrad was calculated and also the percentage of regular tetrads showing no trace of chromosome elimination. The data obtained are given in Table 3.

As is evident from the table, there is a rather pronounced degree of

TABLE 3. *Frequency of micronuclei in the tetrads of tetraploid rye.*

Plant number	Steel-rye		Crossing-group		Sangaste		Toivo	
	a	b	a	b	a	b	a	b
1	0,25	83,3	0,64	65,0	0,36	81,5	0,48	72,8
2	0,16	89,0	0,30	83,0	0,41	78,1	0,33	79,0
3	0,54	77,0	0,40	72,9	0,53	71,0	0,55	68,0
4	0,46	73,0	0,13	89,5	0,51	78,0	0,83	69,0
5	0,79	60,0	0,65	68,0	0,99	49,5	0,38	80,0
6	0,35	80,0	0,35	83,1	0,63	69,0	0,52	67,0
7	1,34	34,0	—	—	0,42	81,0	0,74	59,0
8	1,11	47,0	—	—	0,47	79,1	0,53	73,0
9	—	—	—	—	—	—	0,63	57,0
10	—	—	—	—	—	—	0,16	88,3
11	—	—	—	—	—	—	0,48	74,3
12	—	—	—	—	—	—	0,27	82,9
Average	0,63	67,9	0,41	76,9	0,54	73,4	0,49	72,5

a = average number of micronuclei per tetrad.
b = per cent regular tetrads.

variation within the varieties. The differences between the corresponding average values of the four varieties are not significant. An important factor when considering the variation is the spontaneous aneuploidy. Thus, plant No. 8 of Steel-rye had $2n = 29$, which is reflected in a high average number of micronuclei (1,11) and a low percentage of regular tetrads (47,0). Of the other plants of this variety numbers 2 and 6 were controlled to have $2n = 28$ and are also rather regular in the tetrads. Of the other plants No. 7, having 1,34 micronuclei and only 34 per cent regular tetrads is certainly aneuploid. The remaining four plants are probably euploid. In the crossing-group plant No. 6 was controlled to have $2n = 28$. The other 5 plants may also be euploid. In Sangaste plant No. 8 had 28 chromosomes. The other plants may also be euploid with the exception of plant No. 5. In this plant the average number of micronuclei was as high as 0,99 and the percentage of regular tetrads as low as 49,5. In Toivo, finally, plant No. 12 is the one in which meiosis was studied and the number of chromosomes found to be 28. Most or all of the other plants may also be euploid, but there is a good deal of continuous variation possibly including some aneuploids.

The five plants controlled to be euploid had an average of 0,32 micronuclei and 82,8 per cent regular tetrads, the corresponding value of the single plant controlled to have $2n = 29$ being 1,11 and 47,0. As

most other plants showed more irregularity than the average of the 5 controlled euploids, they certainly include some aneuploids.

More data are needed, but already now it is rather clear that such studies of the tetrads fairly well indicate the degree of meiotic irregularity and the frequency of aneuploids in the material.

2. SOMATIC CHROMOSOME NUMBERS.

As in all other autopolyploids with multivalents and univalents at meiosis tetraploid rye shows a certain variation in chromosome number. The data available on this point are summarized in Table 4. As may be seen from the table, the somatic chromosome numbers range from 26 to 31. The predominant number is 28 and, on an average, this number was found in 77,23 per cent of the 808 plants examined. The next numbers in frequency are 29 and 27, these numbers being represented by 13,12 and 7,43 per cent, respectively. Plants with 26 and 31 chromosomes are quite rare and were so far only observed in single cases.

It is obvious that the hyperploid aberrants are more frequent than the hypoploid ones. The total series at the bottom of Table 4 includes 61 hypo- and 123 hyperploid aberrants. This cannot represent a 1 : 1 distribution as demonstrated by a χ^2-test. This gave a χ^2 of 20,89 and a P much smaller than 0,001. Of the 8 different categories studied all but one (No. 1) showed a higher percentage of hyper- than hypoploids.

With one exception the different categories show about the same frequency of aneuploid aberrants. Thus, the total average values of American, Swedish $\times$ American and Steel-rye are 20,73; 20,39 and 22,71 respectively. Category 8, representing F_1-hybrids between Steel and American had also a similar value, 23,53. Wasa II, on the contrary, is clearly deviating. It shows a total absence of hypotetraploids, and in spite of that the total frequency of aneuploids is as high as 37,14. The standard error of this value is 5,78 and that of Steel-rye (total series) 2,91. The difference is $37{,}14 - 22{,}71 = 14{,}43 \pm 6{,}47$. This gives a t of 2,23 and a P intermediate between 0,05 and 0,02. A χ^2-test concerning the heterogeneity of the same two series will be reduced to a comparison between the ratios 176 : 31 (Steel-rye) and 44 : 26 (Wasa II). This gives a χ^2 of 15,73 and a P smaller than 0,001. A comparison between Wasa and the other categories would evidently give similar values. A computation of the heterogeneity of the entire material would have been more correct, but the calculations undertaken are sufficient to show that the material of Wasa II examined in 1942—1943 showed a higher percentage

TABLE 4. *Chromosomal variation in different strains of tetraploid rye.*

Category	Variety and year	2*n* of mother plants	Chromosome number in offspring						n	Percentage of aberrants		
			26	27	28	29	30	31		Subtetra-ploid	Super-tetraploid	Total
1	American, 1939	28		5	53	4			62	8,06	6,45	14,52
2	» , 1942	?		25	207	28	6		266	9,40	12,78	22,18
1 + 2	» , total	—		30	260	32	6		328	9,15	11,59	20,73
3	Swedish × Am. 1942	?		13	121	18			152	8,55	11,84	20,39
4	Steel-rye, 1942	28		9	89	18	3		119	7,56	17,65	25,21
5	» , 1943	?	1	6	71	9	1		88	7,95	11,36	19,32
4 + 5	» , total	—	1	15	160	27	4		207	7,73	14,98	22,71
6	Wasa II, 1942	28			5	2			7	—	—	—
7	» , 1943	?			39	18	5	1	63	0,00	38,10	38,10
6 + 7	» , total	—			44	20	5	1	70	0,00	37,14	37,14
8	Steel × Am. 1943	?		2	39	9	1		51	3,92	19,61	23,53
1 — 8	Total of all values	—	1	60	624	106	16	1	808	7,55	15,22	22,77

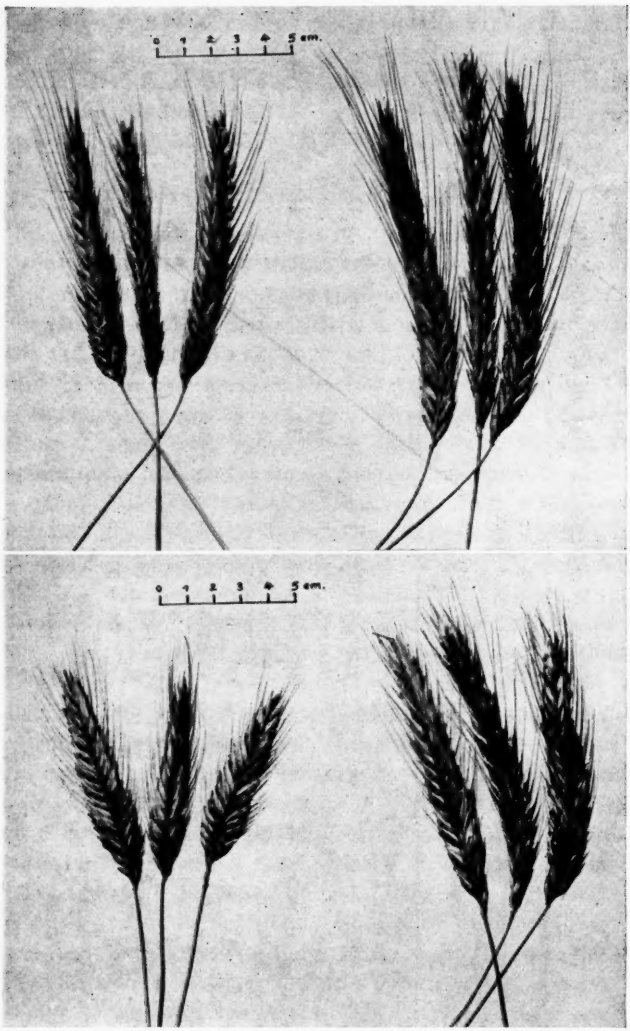


Fig. 13. Ears of Steel-rye (above) and King-rye (below). Diploids to the left, tetraploids to the right.

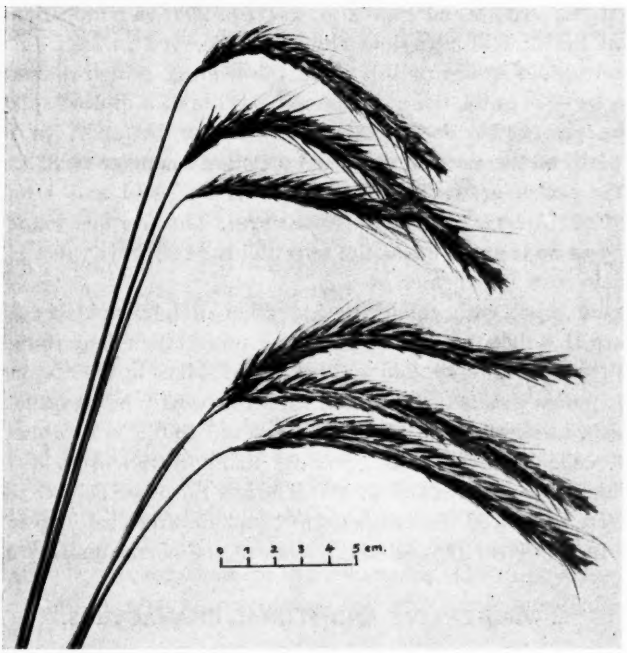


Fig. 14. Ears of Toivo-rye, diploid (above) and tetraploid (below).

of aberrants and a stronger predominance of the hyperploids than the other categories. A new comparison in this respect between Wasa II and other tetraploid strains is evidently desirable.

II. COMPARISON BETWEEN DIPLOID AND TETRAPLOID RYE.

In the main, autotetraploid rye shows the same characteristics, when compared with the corresponding diploids, as most other plant species in which such comparisons have been undertaken (*cf.* MÜNTZING, 1936). Ears of 3 diploid rye varieties and their corresponding tetraploids are shown in Figs. 13—14. However, especially as the changes owing to polyploidy also affect the practical properties of tetraploid rye, it is desirable to present the data available in some detail.

1. CELL SIZE.

Some pollen measurements were undertaken and indicate that the tetraploids have larger pollen grains. These data have already been

published (MÜNTZING and PRAKKEN, 1941, p. 295) in connection with a report on the diploid-tetraploid chimaera observed in Östgöta gråråg. In two tetraploid spikes of this plant the average pollen diameter was found to be 40,16 units, the corresponding value of a diploid spike being 32,79. The former value is 22 per cent larger than the latter. An ordinary diploid plant of the same variety had a pollen diameter of 33,24. At the same time pollen grains were measured in 4 diploid and 4 tetraploid plants of the American variety (Rosen-rye). The average value of the diploids was 35,19 and that of the tetraploids 41,67. This value is 18 per cent larger than the former one.

During work with colchicine induction of tetraploids in Steel-rye and Wasa II pollen measurements were undertaken and found to be of value for separating diploid and tetraploid spikes in the c_0-generation, the large pollen grains of tetraploid spikes generally being quite easy to distinguish. Later on, however, such work was found to be unnecessary, the difference in kernel size generally being sufficient to distinguish tetraploids from diploids (*cf.* p. 41). Though no other cells than pollen grains were measured it seems safe to conclude that also in this species cell size in the tetraploids is larger than in the corresponding diploids.

2. VEGETATIVE AND FLORAL CHARACTERS.

A. PLANT HEIGHT.

The first tetraploid rye cultivated at Svalöf was derived from the American Rosen-rye. In the summer of 1938 it was represented by 13 tetraploid plants growing side by side with the corresponding diploid strain. Height measurements were undertaken but showed no significant difference between diploids and tetraploids. No further comparisons between $2x$ and $4x$ plants of this variety were undertaken, as the cultivation of diploid Rosen-rye had to be stopped from want of space for isolation groups. New opportunities for comparisons between corresponding diploid and tetraploid strains were obtained in 1941 when tetraploids of Steel-rye and Wasa II had been produced. However, as will be explained in detail below, it was soon realized that diploid and tetraploid rye cannot be cultivated side by side in field trials, owing to intercrossing, which spoils the yield figures. Consequently, diploids and tetraploids had to be tested in different fields, using two wheat varieties, Skandia III and Ergo, as common standards.

In the years 1947—1949 height measurements were also undertaken in these field trials, one row of each plot being harvested separately.

TABLE 5. *Plant height in diploid and tetraploid rye.*

Year	Value	Steel-rye			Wasa II			Sangaste			Wheat standards	
		dipl.	tetrapl.	tetr./ dipl.	dipl.	tetrapl.	tetr./ dipl.	dipl.	tetrapl.	tetr./ dipl.	diploid trial	tetrapl. trial
1947	Absolute	93,0	102,6	—	101,6	109,6	—	—	—	—	93,2	92,3
1947	Relative	99,8	111,1	1,11	109,0	118,7	1,09	—	—	—	100,0	100,0
1948	Absolute	141,3	148,8	—	141,3	145,0	—	—	—	—	131,9	122,5
1948	Relative	107,1	121,4	1,13	107,1	118,4	1,11	—	—	—	100,0	100,0
1949	Absolute	101,3	132,1	—	111,3	128,8	—	118,8	132,5	—	111,9	111,9
1949	Relative	90,5	118,0	1,30	99,4	115,1	1,16	106,1	118,4	1,12	100,0	100,0

The plants in this row were studied especially with regard to fertility, but the height of the bunch of plants was also determined before making the other measurements. As each variety was represented by 4 replications (or 5 in 1947), the same number of height values was obtained for diploids as well as tetraploids. The average values were calculated and compared with the height values of the wheat varieties. Giving the average height of the two wheat standards the value 100,0, relative height values were calculated for the rye strains, and finally the relative height values of the corresponding diploids and tetraploids were compared.

The data available are summarized in Table 5. Steel-rye and Wasa II could be studied in all three years, whereas data for Sangaste are only available from 1949. To take an example, the average height (in cm.) of diploid Steel-rye was 101,3 in 1949, the corresponding average of the wheat standards being 111,9. If the latter figure is given the value 100,0 the relative value of the diploid Steel-rye will be 90,5. In the tetraploid field trial the same wheat varieties happened to have the same absolute value 111,9 cm. as in the diploid trial. The absolute height of tetraploid Steel-rye was 132,1 cm., giving a relative value of 118,0. The quotient 118,0 90,5 is 1,30, and, thus, in this case the tetraploids were 30 per cent taller than the corresponding diploids. Table 5 contains six other analogous comparisons, and without exception the tetraploids were again found to be taller than the diploids, the percentage values in these cases ranging from 9 to 16. Thus, though no statistical treatment of the data has been undertaken, the evidence available certainly shows that the tetraploids are taller than the diploids, the total average difference being about 15 per cent.

Direct comparisons in which diploids and tetraploids are grown in

the same field trials are of course necessary, and a small trial of this kind, involving diploid and tetraploid Steel-rye, was observed last summer, but the plants have not yet been measured. In two previous years small plots of these types were also grown side by side for demonstration and for crossing purposes. In all these cases the tetraploids were taller than the diploids.

B. STRAWSTIFFNESS.

As tetraploid rye is somewhat taller than diploid rye the strawstiffness of the tetraploids might be expected to be less good than in the diploids. However, the straw of the tetraploids is thicker, and therefore, in most cases, they are able to remain erect just as well as the diploids. Data on this point from 1948—49 are available from the branch stations of the Seed Association. In all cases the degree of lodging was estimated, using a scale from 0 to 10. Low values indicate high degrees of lodging. Seven sets of data are available, each including a diploid and a tetraploid trial, having one or two wheat standards in common. In most cases the variety Ergo was used as a standard. The absolute values obtained from these trials were the following:

	Degree of strawstiffness												
	2	3	4	5	6	7	8	9	10	11	n	M
Dipl. Steel-rye			1	—	—	—	1	1	2	1	6	8,17
Tetr. »			1	1	—	1	—	1	2		6	7,00
Dipl. Wasa II	1	1	—	—	—	2	—	2	1		7	7,21
Tetr. »	1	1	—	1	1	1	—	2			7	6,36
» crossing-group	1	—	—	1	—	2	1	2			7	7,21
Ergo-wheat			1	2	—	1	1	4	4		13	8,58
Gluten- »					1	—	—	1	2		4	9,25
Odin- »									2		2	(10,50)
Vg 01520-wheat									2		2	(10,50)

Each variate in the table represents an average value of at least 4 replications. The class 10—11 includes values representing the maximal strawstiffness, 10,0.

These absolute values show that, on an average, the wheat varieties have a better strawstiffness than the rye material. It also seems to indicate that the tetraploid rye varieties are slightly inferior to the corresponding diploids. It is obvious, however, that this possible difference is not significant, variation being very marked within the series.

A more accurate comparison could be made by calculating the relative values (wheat standards=100,0). This gave the following result:

	Strawstiffness. Relative values (wheat standard = 100,0)																			n	M
	30	-	40	-	50	-	60	-	70	-	80	-	90	-	100	-	110	-	120		
Dipl. Steel ..							1	—	—		1		3		1					6	88,3
Tetr. » ..					2		—	—	—		1		2		—		—		—	6	85,0
Dipl. Wasa II							2	—		1		3		1						7	76,4
Tetr. »			2		—		—		1		—		1		2		—		—	7	76,4
» cross.-group ...	1		—		—		1		—		2		2		—		—		—	7	90,7

	130	-	140	-	150	-	160	-	170-180
Dipl. Steel ..									
Tetr. » ..	—		—		1				
Dipl. Wasa II									
Tetr. »	1								
» cross.-group ...	—		—		—		—		1

All average values are below 100, showing again that strawstiffness in the wheat standards was better than in the rye varieties. Evidently no differences between the different rye categories are significant, but Wasa II seems to be somewhat inferior to Steel-rye. There are no clear differences in strawstiffness between diploids and tetraploids.

C. DEGREE OF TILLERING.

Though in the first place attention was concentrated on yield and fertility, the data available from the years 1943 to 1949 may also to some extent be used for calculations concerning the degree of tillering. Thus, in 11 different field trials diploid and tetraploid Steel-rye were directly compared and the number of tillers counted either after the harvest or just before flowering. The data obtained are summarized in Table 6. In ten of the eleven experiments the diploid plots showed better tillering than the tetraploids and, on an average, the degree of tillering in the tetraploids was 12,0 per cent lower than in the diploids. The simplest way to demonstrate the significance of this difference is to

TABLE 6. *Degree of tillering in diploid and tetraploid Steel-rye.*

Year	Experiment	Average number of tillers in		Tetrapl. value in per cent of dipl. value
		diploid	tetraploid	
1943	1	277	292	105,4
1944	2	727	686	94,4
1945	3	2557	2204	86,2
1945	4	788	723	91,8
1947	5	1200	985	82,1
1947	6	1343	1235	92,0
1948	7	404	354	87,6
1948	8	479	356	74,3
1948	9	179	143	79,9
1949	10	722	642	88,9
1949	11	1179	1013	85,9

compare the ratio 11 : 1 with the ratio 6 : 6, which would be expected if there were no difference between diploids and tetraploids. A χ^2-analysis in this case gives a $\chi^2 = 8{,}33$ and a P-value intermediate between 0,01 and 0,001. Though the data available are sufficient to show that there is a significant difference, the calculated size of this difference, 12,0 per cent, represents a relatively rough estimation, the different values in Table 6 being obtained from experiments of rather variable size and reliability. More precisely the type of the 11 experiments may be indicated as follows:

Exp. 1: Sown with marker boards (1 kernel per hole), 3 replications, 150 kernels per plot,

Exp. 2: Sown with marker boards (1 kernel per hole), 4 replications, 600 kernels per plot,

Exp. 3: Sown with marker boards (2 kernels per hole), 3 replications, 2400 kernels per plot,

Exp. 4: Sown with marker boards (1 kernel per hole), 3 replications, 150 kernels per plot,

Exp. 5: Sown with marker boards (2 kernels per hole), 1 replication, separate rows examined before flowering,

Exp. 6: Sown with marker boards (2 kernels per hole), 1 replication, separate rows examined before flowering,

Exp. 7: Sown with machine, 1 replication, separate rows examined before flowering,

Exp. 8: Sown with machine, 2 replications, separate rows examined before flowering,

Exp. 9: Sown with marker board (2 kernels per hole), 1 replication, 300 kernels per plot,

Exp. 10: Sown with marker board (2 kernels per hole), 1 replication, 450 kernels per plot,

Exp. 11: Sown with machine, 1 replication, separate rows examined before flowering.

In all these experiments diploids and tetraploids were grown in the same field trial, and in cases with only one replication they were grown side by side. In three cases data from indirect comparisons with the help of common wheat standards are also available. In two of these cases diploid Steel-rye had higher values than the corresponding tetraploids.

Similar though less comprehensive data are available also for diploid and tetraploid Wasa II. In six cases these two types were compared in the same field trial, and in four cases data were gathered from indirect comparisons with the help of wheat standards. In the former

cases the diploid was superior 4 times, the tetraploid 2 times. In the indirect comparisons 2 trials were in favour of the diploids, 2 in favour of the tetraploids. However, more extensive data will probably demonstrate that also in Wasa II the degree of tillering is significantly higher in the diploid than in the tetraploid.

In various trials there was also an opportunity to compare tetraploid Steel-rye and the other tetraploid types with regard to degree of tillering. The results of such comparisons are evident from the following relative values, calculated on the basis of tetraploid Steel-rye = 100.

American	Swedish × American	Toivo	Wasa II	Steel × Wasa II	Steel × American	Crossing-group
70,2	53,8	72,9	86,3	91,1	87,2	92,3
73,2	83,8	53,9	91,5	98,7	83,2	96,4
77,4	74,5		100,8	101,2		95,2
78,4	78,7		91,1	97,0		97,2
			97,5	98,4		91,6
			94,2			
			110,2			
Average: 74,8	72,7	63,4	95,9	97,3	85,2	94,5

Almost without exception these tetraploids have been found to be inferior to tetraploid Steel-rye with regard to the degree of tillering. This is especially true of »Swedish × American», »American» and »Toivo», in which the relative values are as low as 72,5, 74,8 and 63,4 respectively. Steel × American, a cross population tested a few years, is evidently intermediate between the parent strains. The average value is 85,2, the corresponding figures of the parent strains being 100,0 and 74,8. The crossing-group, Wasa II and Steel × Wasa II have better tillering, their average values being only slightly below that of Steel-rye. However, of the 17 relative values representing these three categories only 3 are higher than 100. The chance that 14 : 3 might represent a 1 : 1 distribution is intermediate between 0,01 and 0,001 ($\chi^2 = 7{,}12$). Hence, it is highly probable that at least one of these three categories is significantly inferior to Steel-rye.

The data just discussed were obtained from various experiments at Svalöf including those specified on page 34. It may be superfluous to go into more details. The data available are sufficient to show that the degree of tillering is less good in tetraploid than in diploid Steel-rye but that among the tetraploids and under the conditions at Svalöf Steel-rye is superior or equal to all the other strains tested.

D. NUMBER OF FLOWERS PER SPIKE.

Data on the number of flowers per spike are available from the years 1944, 1945, 1947, 1948 and 1949. These values were in part obtained from field trials, in which diploids and tetraploids could be directly compared, in part from indirect comparisons in which the two categories were grown in different fields. During this work the number of spikelets per spike was counted and not the actual flower number. As there are seldom more than two flowers per spikelet, the number of flowers was considered to be equal to twice the number of spikelets. In reality the flower number is slightly higher, a few spikelets sometimes containing more than two flowers. This error, however, is rather insignificant and certainly does not influence the conclusions which could be drawn from these counts.

Considering first the relations between diploid and tetraploid Steel-rye, the first data were obtained in 1944 from a 4×4 latin square trial. Besides diploid and tetraploid Steel-rye this trial also included two other tetraploids (American and Swedish $\times$ American). From each plot a random sample of 25 spikes was taken and the average number of flowers per spike was calculated. The four plots of diploid Steel-rye gave the average values 67, 71, 69 and 69, respectively, the corresponding values of tetraploid Steel-rye being 62, 62, 61 and 59. According to these values, the number of flowers per spike was 11,6 per cent lower in the tetraploid than in the diploid, the above two series having the mean values 69,0 and 61,0, respectively. The difference between these values is significant. A latin square analysis of the usual type revealed the presence of a significant heterogeneity between the four varieties, the v^2 quotient between the mean squares of »varieties» and »error» being 26,64 and P smaller than 0,001. The standard error of the varieties was found to be 1,21, and consequently the difference between the two average values in question, 69,0 and 61,0, will be $8{,}0 \pm 1{,}71$. This gives a t-value of 4,68 and a P (for 6 degrees of freedom) intermediate between 0,01 and 0,001.

Further evidence of a difference between diploids and tetraploids with regard to the number of flowers per spike was obtained in the following year, 1945. In this year the material was tested partly in a small 3×3 latin square, including diploid and tetraploid Steel-rye and the tetraploid Swedish $\times$ American rye, partly in a larger trial, including six varieties. In the small trial the number of flowers was determined from random samples of 50 spikes, in the large trial from samples of 100 spikes. In the small trial diploid Steel-rye had the values

72,4, 71,6 and 72,2 (mean value: 72,67), the corresponding values of tetraploid Steel-rye being 67,8, 69,5 and 65,6 (mean value: 67,67). Thus, again the diploid had a larger number of flowers, the difference in this case being 6,3 per cent.

However, as revealed by a latin square analysis this difference *per se* is not significant but points in the same direction as before. This was also repeated in the larger trial of 1945, diplod and tetraploid Steel-rye in this case having the average values 67,98 and 60,98, respectively. This corresponds to a difference of 10,6 per cent. Further direct comparisons were made in 1948 and 1949 on material from single plots of diploid and tetraploid Steel-rye, growing side by side. In 1948 the average values 51,62 (dipl.) and 48,80 (tetrapl.) were obtained, giving a percentage difference of 5,5. In 1949 the corresponding values were 51,99 (dipl.), 49,42 (tetrapl.) and 4,9 per cent less flowers in the tetraploid than in the diploid.

The indirect comparisons are of course less reliable, but it may be mentioned that in all three years 1947 to 1949 tetraploid Steel-rye gave somewhat lower values than the corresponding diploid. In 1947 the absolute values were 42,2 (dipl.) and 41,3 (tetrapl.), in 1948 they were 50,8 (dipl.) and 45,5 (tetrapl.). Relative values (in relation to the average number of spikelet per ear in the two wheat varieties used as standards) were also calculated. With the use of these relative values it was found that the deficit of flowers per spike in tetraploid Steel-rye was 17,7 per cent in 1947 and 16,7 per cent in 1948.

The data from 1949 are of more value and represent a new aspect of the problem. It had been observed repeatedly that in tetraploid as well as in diploid rye there is a tendency of the basal spikelets to fall off, and, thus, at the time of harvest when the spike is ripe, the number of spikelets is generally lower than in the young spike just appearing. This shedding of some of the spikelets will reduce the yield and therefore deserves careful attention.[1] It was soon realized that the tendency to shedding is somewhat more pronounced in the tetraploids than in the corresponding diploids. As the scars left by the spikelets shed are quite easy to count, the number of spikelets formed as well as the number remaining at the spike could be determined. The data obtained in 1949 are summarized in Table 7.

The diploid and tetraploid rye material were grown in different

[1] This work was stimulated by similar counts previously undertaken by Dr. ERIK ÅKERBERG and his assistant Mr. SCHILDT. I am much indebted to these gentlemen for showing me their results.

TABLE 7. *Number of flowers per spike. Diploid and tetraploid rye 1949.*

Variety	Number of flowers formed (spikelets in wheat standards)		Numb. of flowers in mature spikes (spikelets in wheat standards)		Difference 2—4	Decrease in per cent of 2	Decrease, rel. values
	Absolute	Relative	Absolute	Relative			
1	2	3	4	5	6	7	8
Steel-rye, dipl.	52,78	288,4	46,98	334,6	5,80	10,99	46,9
Wasa II, »	47,78	261,0	45,20	321,9	2,58	5,40	23,1
Sangaste, »	50,83	277,8	45,40	323,4	5,43	10,68	45,6
Ergo, wheat	19,75	100,0	15,48	100,0	4,27	21,62	100,0
Skandia III, wheat	16,35		12,60		4,25	25,22	
Steel-rye *a*, tetr.	48,88	267,4	46,43	343,4	2,45	5,01	19,2
» *b*, »	47,28	258,6	39,38	291,3	7,90	16,71	64,1
» *c*, »	50,08	274,0	42,03	310,9	8,05	16,07	61,6
Crossing-group, tetr.	46,00	251,6	39,23	290,2	6,77	14,72	56,4
Wasa II, tetr.	43,38	237,3	35,20	260,4	8,18	18,86	72,3
Steel × Wasa II, tetr.	46,25	253,0	39,60	292,9	6,65	14,38	55,1
Sangaste, tetr.	41,80	228,7	36,38	269,0	5,42	12,97	49,7
Ergo, wheat	19,05	100,0	14,23	100,0	4,82	25,30	100,0
Skandia III, wheat	17,50		12,80		4,70	26,86	

fields, using the two wheat varities Ergo and Skandia III as common standards. The diploid trial included the rye varieties Steel, Wasa II and Sangaste. The tetraploid trial comprised seven different rye types. Three of these, Steel-rye *a*, *b* and *c*, are closely related, *a* and *b* representing two different populations propagated in Scania (Ahleborg and Svalöf) and *c* a population cultivated a few years at Flahult in the province of Småland.

The absolute values given in Table 7 (columns 2 and 4) are average values of four replications. Each of these four values are based on counts of all spikes in one particular row of the plot, this row being harvested separately. On an average the row contained more than 200 spikes. Thus, the data in Table 7 are based on a rather extensive material.

In the wheat standards only the number of spikelets per ear was counted. The average number of the two varieties was compared with the number of flowers per ear in the rye varieties and relative values calculated.

Considering first the number of flowers formed, the absolute values in column 2 may be directly compared, the average values of the wheat standards in this case being practically the same in the diploid and tetraploid trials (18,30 and 18,28, respectively). In all three rye varieties represented by diploids as well as tetraploids the number of flowers per spike was slightly higher in the diploids than in the tetraploids. Comparing the absolute and relative average values of tetraploid Steel *a*, *b* and *c* with those of diploid Steel, the average number of flowers formed was found to be 7,5 per cent lower in the tetraploid than in the diploid. The corresponding values in Wasa II and Sangaste are 9,1 and 8,9 per cent, respectively.

At the time of harvest all categories showed a lower number of flowers per spike, the reduction in absolute number being given in column 6. Calculating the decrease in per cent of the original number (column 7), it was found that the decrease ranged from 5,40 to 10,99 in the diploid rye varieties and from 5,01 to 18,86 in the tetraploids. There was also a considerable degree of shedding of the spikelets in the wheat standards, this shedding being somewhat more pronounced in the standards of the tetraploid trial than in those of the diploid trial. Calculating relative values for the degree of reduction (wheat standards = 100,0), these values were somewhat higher in the tetraploids than in the corresponding diploids. Thus, the average reduction value of tetraploid Steel-rye is 48,3, the corresponding value of diploid

Steel being 46,9. In Wasa II the values to be compared are 72,3 ($4x$) and 23,1 ($2x$), in Sangaste 49,7 ($4x$) and 45,6 ($2x$).

The value of tetraploid Steel-rye just given, 48,3, is the average of three values, 19,2, 64,1 and 61,6, of which the first one differs significantly from the other two. The occurrence of this difference is interesting, as Steel-rye *a* differed from Steel-rye *b* and *c* by having a lower number of spikes per plot. The average numbers of spikes were 166,5 (*a*), 203,5 (*b*) and 197,7 (*c*). The reason of this difference was that *a* in contrast to *b* and *c* had been severely damaged by too much dressing of the seeds with fungicides before sowing. Consequently, germination in *a* was quite poor, and only by and by the plants were able to compensate the original loss to a certain extent by a higher degree of tillering of the individual plants. However, in spite of this the definite result was a less dense stand in *a* than in *b* and *c*. Evidently, this was favourable to the development of the ears, the shedding of the basal spikelets being clearly less pronounced in *a* than in the other representatives of tetraploid Steel-rye. Thus, in reality the difference in degree of shedding between diploid and tetraploid Steel must have been more pronounced than indicated by the figures just cited.

Some additional data were obtained in the same year from two plots of Steel-rye, one diploid and one tetraploid, growing side by side in another field trial, chiefly comprising varieties of *Triticale*. These plots had been sown with marker boards (2 kernels per hole), and had about the same density as the plots sown with machine. Counts of a total of 2006 spikes from the entire plots gave the following values:

	Diploid	Tetraploid
Number of flowers formed	55,52	54,88
» » » in mature spikes ...	51,99	49,42

The differences are evidently very slight but again indicate that the values are less favourable in the tetraploid than in the diploid.

Surveying all data presented above we have found, in direct as well as indirect comparisons, that the number of flowers in the mature spikes is always slightly lower in the tetraploids than in the corresponding diploids. A similar though less pronounced difference is met with in the young spikes in which no shedding has yet occurred. The reduction by shedding is more pronounced in tetraploids than in diploids and more pronounced in dense stands than in plots with a relatively low number of spikes.

These conclusions are based on data only part of which have been

subject to a detailed statistical treatment. However, the validity of the conclusions is demonstrated by the fact that in all of 5 direct and 3 indirect comparisons diploid Steel-rye was found to be superior to the corresponding tetraploid. The probability that such a difference, repeated 8 times, is insignificant is as low as $(^1/_2)^8 = 0{,}004$. It is probably characteristic also of other tetraploids, 4 sets of data involving Wasa II (3 comparisons) and Sangaste (1 comparison) giving analogous results.

So far the exact degree of reduction is difficult to state. The most reliable value is 7,7 per cent for tetraploid Steel-rye from direct comparisons. The indirect comparisons gave a higher average value, 14,8 per cent, and for Wasa II and Sangaste the data available were still less favourable for the tetraploids. In Wasa II the number of flowers in the mature spikes was 24,2 per cent lower than in the diploid, the corresponding value of Sangaste being 16,8.

Naturally, the environmental conditions in different years will greatly influence the degree of shedding. However, the average value 7,7 per cent for Steel-rye, based on direct comparisons in 5 different years, probably represents a fairly reliable value.

Finally, it remains to be said that without exception tetraploid Steel has been found to be superior to all the other tetraploid rye strains tested simultaneously. This is evident from the following relative values (tetraploid Steel-rye = 100,0).

Year	American	Swedish × American	Steel × American	Steel × Wasa II	Wasa II	Sangaste	Crossing-group
1944	88,9	95,5					
1945 a	82,8	94,0	96,6		98,2		
1945 b		96,3					
1947				96,1	97,3		91,5
1948				99,4	92,6		89,5
1949				97,3	86,5	89,4	96,4
Average:	85,9	95,3	(96,6)	97,6	93,7	(89,4)	92,5

These values refer to the number of flowers in the mature spikes. Steel × Wasa II is only slightly less good than Steel, but the average value of this hybrid population, 97,6, is evidently intermediate between those of the parent strains (Wasa II: 93,7 and Steel: 100,0).

E. KERNEL SIZE.

In all tetraploid rye strains so far studied, kernel size was found to be considerably larger than in the corresponding diploids. This is

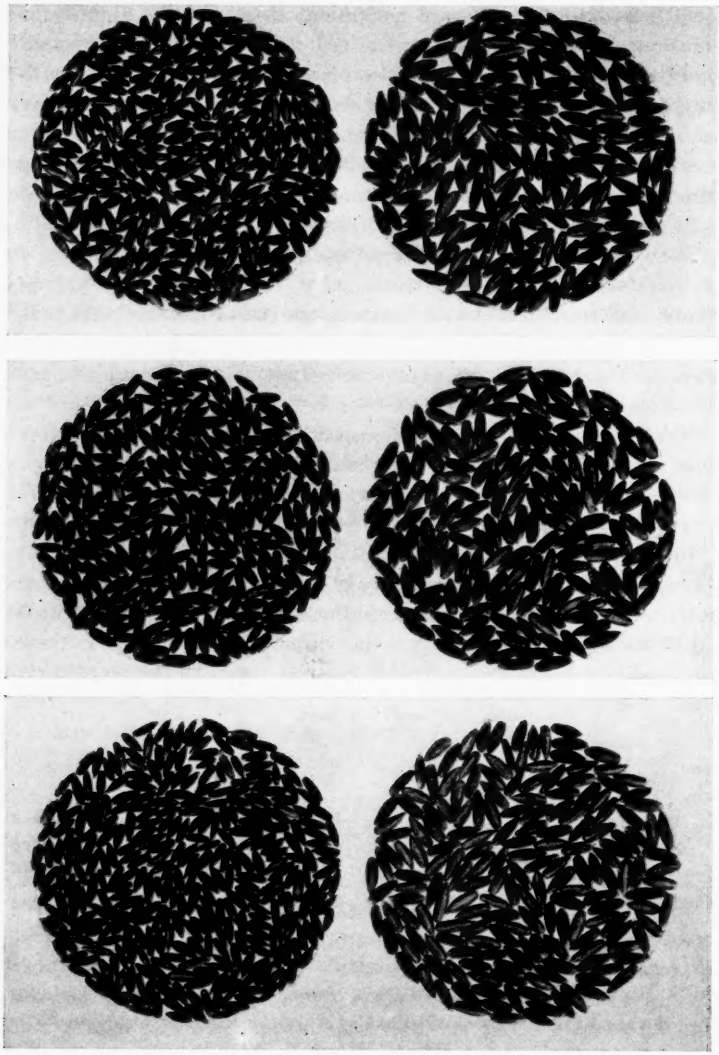


Fig. 15. Seed samples of Steel-rye (above), King-rye (in the middle) and Wasa II (below). Diploid samples to the left, tetraploid to the right.

TABLE 8. *Thousand grain weight in diploid and tetraploid Steel-rye.*

Trial No.	Place of trial	Year	Absolute values (gr.)			Relative values		
			Dipl.	Tetr.	Tetr. in % of dipl.	Dipl.	Tetr.	Tetr. in % of dipl.
1	Svalöf	1945	31,2	48,2	154,5	—	—	—
2	»	1945	32,0	46,1	144,1	—	—	—
3	»	1946	34,4	41,2	119,8	72,5	94,5	130,3
4	»	1947	32,2	47,4	147,2	83,1	116,6	140,3
5	»	1948	26,7	43,2	161,8	64,1	109,8	171,3
6	»	1948	29,4	47,7	162,2	—	—	—
7	»	1949	29,5	53,0	179,7	63,8	109,2	171,2
7	»	1949	29,5	49,1	166,4	63,8	101,1	158,5
7	»	1949	29,5	49,5	167,8	63,8	102,0	159,9
7 (average)	Svalöf	1949	29,5	50,5	171,2	63,8	104,1	163,2
8	Svalöf	1950	27,9	44,6	159,8	57,3	90,0	157,1
8	»	1950	27,9	45,9	164,5	57,3	92,6	161,6
8	»	1950	27,9	45,0	161,3	57,3	90,8	158,5
8	»	1950	27,9	46,1	165,2	57,3	93,0	162,3
8 (average)	Svalöf	1950	27,9	45,4	162,7	57,3	91,6	159,9
9	Linköping	1947	28,9	50,8	175,8	62,9	104,2	165,7
10	»	1948	31,9	51,8	162,4	70,1	111,3	158,8
11	»	1949	29,6	44,3	149,7	66,4	99,6	150,0
12	Skara	1948	30,3	46,2	152,5	72,3	103,4	143,0
13	»	1949	30,8	46,6	151,3	64,8	102,8	158,6
14	Ultuna	1948	25,0	46,2	184,8	64,6	106,9	165,5
15	Varpnäs	1949	34,6	41,8	120,8	84,1	127,1	151,1
Average:			30,29	46,49	153,48	68,83	105,99	153,99

illustrated by Fig. 15, showing kernels of diploid and tetraploid Steel-rye, Wasa II and King-rye. Since 1945 determinations of the 1000-grain weight have been carried out at Svalöf as well as at various branch stations of the Swedish Seed Association. All data available with regard to kernel weight in diploid and tetraploid Steel-rye are summarized in Table 8.

In 1945 diploids and tetraploids were grown together and directly compared. Two different trials of this kind gave the average values 31,2 and 32,0 for 1000-grain weight in diploid Steel-rye, the corresponding values in tetraploid Steel-rye being 48,2 and 46,1, respectively. One pair of these values represents the average of four replications, the other pair three replications. The values of the tetraploids are 54,5 and 44,1 per cent higher than those of the diploids.

As explained below (p. 50), diploids and tetraploids were grown in different machine-sown trials from 1946 onwards, using wheat varieties as common standards. Therefore relative kernel weights were also calculated in these cases, *i. e.* kernel weights of diploid and tetraploid rye in per cent of the average kernel weight of the wheat standards. As a rule, two wheat varieties were used as standards (generally Skandia III and Ergo), and in such cases the average of the two wheat standards was used for the comparison. Each wheat variety was replicated four times just as the rye varieties. In some cases the number of replications was as high as 6.

In 1946 the trial at Svalöf gave the average absolute 1000-grain values 34,4 (diploid) and 41,2 (tetraploid), the latter value being 19,8 per cent higher than the former. In this case, however, the diploid material was grown in a more favourable field than the tetraploids, and this explains the relatively small difference in absolute kernel weight between $2x$ and $4x$. The relative values in this case were 72,5 for the diploid and 94,5 for the tetraploid. The latter value corresponds to 130,3 per cent of the former value and, thus, according to the indirect comparison, kernel weight in the tetraploid was 30,3 per cent higher than in the diploid. In all other trials, however, the difference was more pronounced. According to Table 8, the absolute 1000-grain weights ranged from 25,0 to 34,6 in the diploid and from 41,2 to 53,0 in the tetraploid. The tetraploid values expressed in per cent of the diploid ones ranged from 119,8 to 184,8 with an average of *153,48*. Thus, judging from the absolute values kernel weight in tetraploid Steel-rye is 53,48 per cent larger than in diploid Steel.

The relative values, on the other hand, ranged from 57,3 to 84,1 in

the diploid and from 90,0 to 127,1 in the tetraploid. Expressing the latter values in per cent of the former, the increase in kernel weight in 4*x* ranged from 30,3 to 71,3 with an average of *153,99*. Thus, the comparisons between the absolute as well as the relative values gave closely similar results, demonstrating that kernel weight in tetraploid Steel-rye is about 53 or 54 per cent larger than in diploid Steel. This conclusion is based on 15 different trials. In trial No. 7 tetraploid Steel was represented by three different populations, raised at different places, and in trial No. 8 by four different elites or populations. When calculating the total average of the 15 trials, only the average values of trials 7 and 8 were used, not the values of the separate elites and populations.

TABLE 9. *Kernel weight in diploid and tetraploid rye varieties.*

Variety	Thousand grain weight in gr. 24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	n	M
Steel-rye, dipl.		1	2	4	4	2	2										15	30,34
» , tetr.									2	1	2		6	1	3		15	46,60
Wasa II, dipl.		2	—	4	3	2											11	29,55
» , tetr.									2	1	1		3	3	1		11	46,27
Steel-rye × Wasa II, tetr.										1	1	—	1	1	1		5	48,20
Crossing-group, tetr.										1	3		4	1	1	2	12	47,67

Kernel weight in diploid and tetraploid rye is further illustrated by Table 9. The two series for diploid and tetraploid Steel-rye represent the 15 pairs of absolute values given in Table 8. Data for Wasa II were gathered from 11 different trials. Just as in Steel-rye the series representing diploid and tetraploid Wasa II are quite separate and on different levels, the average values being 29,55 and 46,27, respectively. The latter value is 56,58 per cent larger than the former.

Two other tetraploid categories, without corresponding diploids, are included in Table 9. In 5 trials with a hybrid population of the cross Steel × Wasa II the average 1000-grain weight was found to be as high as 48,20, and in 12 trials the tetraploid crossing-group had the corresponding value 47,67. As before, the individual values in Table 9 generally represent averages of four replications.

From all the data in Tables 8 and 9 the conclusion may be drawn that *kernel weight in tetraploid rye is more than 50 per cent larger than in diploid rye.*

TABLE 10. *Hectolitre weight in diploid and tetraploid Steel-rye.*

Trial No.	Place of trial	Year	Absolute values (kg)			Relative values		
			Dipl.	Tetr.	Tetr. in % of dipl.	Dipl.	Tetr.	Tetr. in % of dipl.
1	Svalöf	1949	72,3	70,5	97,5	94,2	90,6	96,2
1	»	1949	72,3	71,1	98,3	94,2	91,3	96,9
1	»	1949	72,3	70,6	97,6	94,2	90,7	96,3
1 (average)	Svalöf	1949	72,3	70,7	97,8	94,2	90,9	96,5
2	Svalöf	1950	77,0	74,4	96,6	98,8	94,8	96,0
2	»	1950	77,0	74,0	96,1	98,8	94,3	95,4
2	»	1950	77,0	73,9	96,0	98,8	94,1	95,2
2	»	1950	77,0	73,9	96,0	98,8	94,1	95,2
2 (average)	Svalöf	1950	77,0	74,1	96,2	98,8	94,3	95,4
3	Linköping	1947	72,2	68,9	95,4	91,0	86,0	94,5
4	»	1948	70,4	64,9	92,2	91,0	85,7	94,2
5	»	1949	75,0	71,3	95,1	93,5	87,7	93,8
6	Skara	1948	72,5	66,8	92,1	93,0	88,4	94,1
7	»	1949	73,2	68,5	93,6	93,6	90,3	96,5
8	Ultuna	1948	71,2	66,5	93,4	92,5	85,0	92,0
9	Varpnäs	1949	75,0	67,0	89,3	96,8	90,0	93,0
10	Kalmar	1950	75,3	73,0	96,0	98,2	92,4	94,1
Average			73,41	69,17	94,20	94,35	89,25	94,59

F. HECTOLITRE WEIGHT.

In the cereals the weight in kilogram of one hectolitre is widely used as an indicator of quality, low hectolitre weights generally being correlated with poor quality. The value of this character is somewhat dubious, it is true, the volume weight not only depending on the quality of the kernels but also on the specific kernel shape and packing ability characteristic of the variety in question. Nevertheless it is of interest to compare the hectolitre weight in the diploid and tetraploid rye varieties. If the cultivation of tetraploid rye should become widely spread, the traditional determinations of hectolitre weight would certainly be carried on in the usual way and, hence, it is necessary to examine to what extent the present quality scales are possible to use on the tetraploid level. As the tetraploid rye kernels are, on an average, more than 50 per cent larger than the diploid ones, this may, indeed, be expected to influence the hectolitre weight rather markedly.

The results actually obtained are summarized in Tables 10—11. With regard to Steel-rye, hectolitre weight was determined in 10 different trials during the years 1947 to 1950 (Table 10). Without exception the tetraploid had lower values than the diploid. This is true of the absolute as well as the relative values. The latter ones were calculated with the help of the wheat standards common to the diploid and tetraploid trials, just as in the case of the 1000-grain weight. Considering first the absolute values, the figures representing the tetraploids in per cent of the diploids range from 89,3 to 97,8 with an average of 94,22. The relative values, obtained with the help of the wheat standards, gave a very similar result, the range of variation of the percentage values being from 92,9 to 96,5 with an average of 94,59. Thus, in tetraploid Steel-rye hectolitre weight is about 5—6 per cent lower than in diploid Steel-rye.

In Table 11 the absolute hectolitre weights of diploid and tetraploid Steel-rye are given as well as the corresponding data of diploid and tetraploid Wasa II, tetraploid Steel × Wasa and the tetraploid crossing-group. The variates in the table are average numbers from different field trials. Thus, diploid and tetraploid Wasa II were examined in 10 trials giving the average values 73,00 and 68,88, respectively. The latter value amounts to 94,36 per cent of the former and, hence, also in Wasa II hectolitre weight in the tetraploid is 5 or 6 per cent lower than in the diploid. The tetraploid crossing-group had about the same average value as the other tetraploids, *viz.* 69,39, and also the remaining tetraploid category, Steel × Wasa, seems to represent the same level. Thus,

TABLE 11. *Hectolitre weight in diploid and tetraploid rye varieties.*

	Hectolitre weight (kg)																		n	M
	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	
Steel-rye, dipl.												1	1	3	1	—	3	—	1	10	73,70
» , tetr.						1	—	2	1	2	—	1	1	—	1	1				10	69,20
Wasa II, dipl.												1	—	4	1	1	1			8	73,00
» , tetr.								2	1	2	—	1	2							8	68,88
Steel × Wasa, tetr.										1	—	—	1							2	(70,00)
Crossing-group, tetr.								2	1	2	1	—	2	—	—	1				9	69,39

it is highly probable that the results obtained are characteristic not only of Steel-rye and Wasa II but of rye in general.

3. FERTILITY.

In rye just as in other plant species fertility is reduced in the autotetraploids, at least with regard to seed setting. This is elucidated by the following data.

A. PERCENTAGE OF SEED SETTING.

Data on seed setting in various tetraploid strains as well as in their corresponding diploids are available for the period 1941 to 1949 and are summarized in Table 12. The variates in this table are average values of generally four replications. The percentages of seed setting have been calculated on the assumption that each spikelet has two flowers. In reality the spikelets may occasionally have more than two flowers, but the error thus made must be very slight and without significance.

The first impression of Table 12 is a rather pronounced variation between the values of different years and varieties as well as between diploids and tetraploids. However, a close study of the table will reveal some significant facts. In the table there are separate columns denoted »Trial» and »Isolation». This means that the percentage of seed setting in most of the cases was not only determined in the isolation groups, in which admixture of foreign pollen was as far as possible prevented by distance isolation, but also in the field trials, in which different varieties were grown together. In both categories seed setting was determined in all spikes of a random sample. In the trials this sample was obtained by a separate harvesting of one or two of the middle rows of the plot, in the generally rectangular isolation groups the sample was

TABLE 12. *Percentage of seed setting in diploid and tetraploid rye varieties.*

Variety	1941	1942	1943	1944		1945		1946		1947		1948		1949
	Isolation	Isolation	Isolation	Trial	Isolation	Trial	Isolation	Trial	Isolation	Trial	Isolation	Trial	Isolation	Trial
Dipl. Steel-rye				60,3	80,3	48,8		81,4		94,1		65,6		71,1
Tetr. »			54,4	44,5	63,0	38,6	55,7	59,8	68,1	73,2	61,6	56,1	66,8	62,7
Dipl. Sangaste														63,8
Tetr. »											59,2		56,6	48,1
Dipl. Wasa II								76,6		88,2		70,8		67,6
Tetr. »					54,6	48,6	55,3	54,8	60,0	68,2	61,3	55,4	61,8	58,3
» Steel×Wasa II							65,1	60,3	63,0	69,6	65,0	54,2	60,6	62,1
» American	29,7	29,0	34,9	37,5	52,5	45,1		56,5	62,4					
» Sw. × Am.		57,9	65,4	43,0	69,1	47,1	55,7	60,5	65,1					
» Steel × Am.					61,7	43,6	55,3	58,6	66,7					
» Crossing-group							54,9	58,9	68,2	75,6	69,6	56,9	62,4	59,4
» Toivo													58,2	

taken by cutting out a narrow band of spikes across the middle of the plot.

In Table 12 a thick vertical line between the years 1945 and 1946 divides the table into two parts. The values to the left of this line are characterized by the fact that in these years (1944 and 1945) diploid and tetraploid rye strains were compared directly, side by side in the same trial. In the years 1946 to 1949 (to the right in the table) diploids and tetraploids were compared indirectly and in different, spatially isolated trials, using wheat varieties as common standards.

The reason for this change in type of field trial is that in 1945 it became quite clear that seed setting in the tetraploids is reduced, if they are exposed to the pollen of ordinary diploid rye. Thus, in 1944 and 1945 seed setting in the field trials, in which the tetraploids were exposed to ordinary rye pollen, was much lower than in the isolation groups, in which the pollen present was only derived from tetraploids. In 1944 tetraploid Steel-rye had a seed setting percentage of 44,5 in the trial and 63,0 in the isolation groups. The corresponding values of »tetraploid American» were 37,5 and 52,5 and of »Swedish × American» 43,0 and 69,1, respectively. In the following year four pairs of values of the same kind were available, all values in the isolation groups being higher than those in the trials.

The data in the right half of Table 12, on the contrary, show no obvious differences in percentage of seed setting between isolation groups and trials, the values of each category sometimes being higher, sometimes lower than those of the other category. There is a total of 15 comparisons of this type, in which the trials only contain tetraploids and their wheat standards. These values as well as the previous 7 pairs, showing the deleterious effect of haploid rye pollen on seed setting in the tetraploids, may be better supervised in the following way:

Percentage of seed setting	35—	40 —	45 —	50 —	55 —	60 —	65 —	70 —	75 —	80	n	M
Trial, 2x and 4x together	2	3	2								7	42,50
Isolation				1	4	1	1				7	58,93
Trial, 2x and 4x separated				2	7	2	2	1	1		15	61,17
Isolation						8	7				15	64,83

The first two series, representing the value from 1944 and 1945, are significantly different as demonstrated by an analysis of variance. For this calculation the exact values given in Table 12 were used instead of the approximate ones resulting from the division into the classes made above. The v^2 value will be 30,37, which corresponds to a P smaller

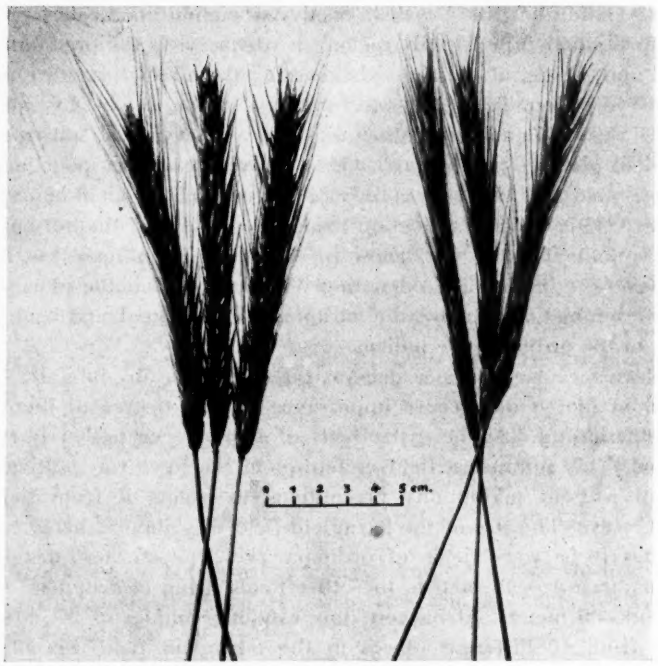


Fig. 16. Ears of tetraploid Steel-rye. To the left from an isolation group, to the right from a plot severely exposed to the pollen of diploid rye.

than 0,001. The exact average values of the two series are 43,28 and 58,09, the difference between these values being 14,81. On the contrary, the other two series, representing the 15 pairs of values of the right part of Table 12 (the years 1946 to 1949) do not differ significantly, the difference between the means only amounting to 3,66 per cent.

Also in recent years I have repeatedly observed the deleterious effects of ordinary rye pollen on the seed setting in the tetraploids. For demonstration purposes I have grown small plots of diploid and autotetraploid rye close together, and in such cases the percentage of seed setting is very markedly reduced in the tetraploids. This is illustrated by the ears to the right in Fig. 16, the ears to the left representing the fairly good seed setting obtained in the isolation groups of tetraploid rye.

A very striking reduction of seed setting in tetraploid Steel-rye was observed in 1945 in the field trials with rye carried out at Ugerup, a

branch station of the Swedish Seed Association in Eastern Scania. Ugerup is situated in a sandy region, in which rye is the predominating cereal and, hence, at the time of flowering the air of the entire area is full of rye pollen. Tetraploid Steel-rye was compared side by side with diploid Steel-rye and some other diploid rye types. Seed setting in the tetraploid plots (4 replications) was observed to be very poor, and the average yield in this case was only 35 per cent of the yield of ordinary Steel-rye. This means a reduction to about one third of the normal yield of tetraploid Steel-rye (*cf.* below p. 65). A contributing cause to this very severe reduction in seed setting was the fact that the plots in this case were long and narrow, the tetraploid plants thus being heavily exposed to the ordinary rye pollen.

More recent experience demonstrates that the absolute size of the tetraploid plot is of decisive importance for the degree of incrossing. With increasing size the evil effects of haploid rye pollen is rapidly reduced. This summer a field of tetraploid Steel-rye was cultivated at Ugerup without taking any precautions to isolate it from fields of ordinary rye. The size of the tetraploid field was about 2 acres, and on all sides there were fields of ordinary rye. The shortest distance to ordinary rye was 48 meters, the other fields being at distances of 125, 200 and 300 meters. At harvest time random samples of 25 ears were taken from 15 different places in the tetraploid field. Six of these samples were taken around the edge of the field and gave the following percentages of seed setting: 61, 63, 65, 63, 51 and 56. The average of this series is 59,8. Six other samples were taken in the field 10 meters from the edge and gave the following values: 66, 61, 62, 67, 61 and 56, the average of this series being 62,2. Three samples, finally, were taken in the middle of the field and had the values 63, 63 and 64 with an average of 63,3. Thus, going from the edge to the interior, the average percentages ranged from 59,8 to 62,2 and 63,3, apparently indicating that seed setting is slightly higher inside the field than at the edges. This difference, however, is not significant, the variance within series being larger than that between series. Further, all the values correspond to normal seed setting in isolation groups of tetraploid rye (Table 12). Under such circumstances the only conclusion to be drawn is that in a tetraploid field of this size and position the pollen of the neighbouring ordinary rye fields has not caused any significant decrease in seed setting. The experiment is now being repeated at Ugerup in a more exact way, the diploid rye closely surrounding the tetraploid central area. Anyhow, the experience already gathered indicates that if the tetraploid

field is not too small it may be placed near ordinary rye without much danger. However, for the time being a distance of about 100 meters may be recommended as sufficiently safe.

The degree of seed setting in tetraploid rye is not only influenced by proximity to diploid rye but also to some extent by the density of the stand. This was evident from data obtained in 1941 and 1942 with the tetraploid American rye. In an isolation group of this variety, sown with the help of marker boards, part of the group was sown with 1 kernel per hole, the other part with 2 kernels per hole. From both these groups a random sample of 200 ears was studied with regard to the percentage of seed setting. In 1941 the following result was obtained:

	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	n	M
1 kernel per hole	7	7	16	26	22	36	33	18	16	4	12	2	—	—	1					200	28,23
2 kernels per hole	3	11	13	16	21	29	31	26	21	12	11	3	—	—	1	1	1			200	31,20

The difference between the average values is 2,97. An analysis of variance of these two series gave a v^2 of 4,8465 and a P-value intermediate between 0,05 and 0,01.

Next year the same experiment was repeated and in addition the percentage of seed setting was also determined in an isolation group of the tetraploid variety »Swedish × American». The following data were gathered:

	Percentage of seed setting																				
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	n	M
Am. 1 k. per hole	6	11	21	26	29	29	24	22	13	9	6	1	2						199	26,87
Am. 2 k. per hole	7	22	24	22	14	14	5	17	14	16	27	9	5	4					200	31,20
Sw.×Am. 1 k. per hole		1	1	2	4	6	9	8	7	11	15	35	30	27	21	13	10		200	57,88

In this year, 1942, the difference between the 1- and 2-kernel series of the tetraploid »American» was 4,33. An analysis of variance gave a v^2 value of 7,43, which corresponds to a P intermediate between 0,01 and 0,001. Thus, the combined data of the two years clearly show that a more dense stand slightly increases the percentage of seed setting.

Differences between tetraploid varieties, however, may be much more striking. According to the above table, the average percentage of

seed setting in »Swedish × American» was about twice as high as that of »American», and in 1943 the same thing was again observed, the average seed setting percentage in the isolation group of »American» being 34,9, the corresponding value of »Swedish × American» being 65,4 (Table 12). In 1944 the difference between the two varieties was less pronounced, the values being 52,5 and 69,1, respectively. In 1946, finally, the American tetraploid seems to have reached the same level of seed setting as the other tetraploid rye varieties. This result may in part be ascribed to intensive selection, but I have also reason to assume that some degree of intercrossing between the American tetraploid and other tetraploid strains may have taken place on account of insufficient spatial isolation. This could not be further studied, as the American tetraploid was discarded in 1946 on account of its low yield in comparison to the other tetraploid varieties. The percentages of seed setting in these varieties have been similar, no differences being significant. More precisely the following values have been obtained (compiled from Table 12):

Variety	Trials	Isolation groups
Steel	62,95 (4)	61,60 (6)
Wasa II	59,18 (4)	58,60 (5)
Steel × Wasa II	61,55 (4)	63,42 (4)
Crossing-group	62,70 (4)	63,78 (4)
Sangaste	48,10 (1)	57,90 (2)
Toivo	—	58,20 (1)
Steel × Am.	58,60 (1)	61,23 (3)
Swedish × Am.	60,50 (1)	62,64 (5)
American	[56,5 (1)]	41,70 (5)

In this survey the varieties just discussed, »American» and »Swedish × American», are also included for comparison. The figures in brackets indicate the number of trials or isolation groups on which the average numbers are based. With regard to the trials only values from 1946 or later are included, seed setting in these trials not being disturbed by the presence of haploid rye pollen.

The only clearly deviating value is that of »American» (isol. groups). Of the other varieties Sangaste, Toivo and Wasa II may have a lower degree of seed setting than the remaining categories, but the data now available are not sufficient to prove that statistically. The ten values of Steel were compared with the nine values of Wasa II, but the analysis of variance gave a v^2 as low as 4,65, which corresponds to a P intermediate between 0,2 and 0,05. Disregarding the American tetraploid,

which is clearly deviating, the general average values of all the other varieties will be 61,12 and, thus, about 60 per cent of seed setting may be said to be characteristic of tetraploid rye in general.

It now remains to consider seed setting in diploid rye and to compare diploids and tetraploids in this respect. As is evident from Table 12, the percentages of seed setting in diploid Steel, Wasa II and Sangaste are rather variable but on an average higher than in the corresponding tetraploids. To elucidate this point the data available in Table 12 may be grouped in the following way:

	Percentage of seed setting												
	45 - 50	- 55	- 60	- 65	- 70	- 75	- 80	- 85	- 90	- 95	n	M
Diploids				1	2	2	1	2	1	1	10	76,50
Corresp. tetraploids	1	1	4	2	1	1					10	59,50
Other tetraploids in the field trials		1	4	3	1	—	1				10	61,50
Tetraploid isol. groups		3	8	8	10						29	61,81

The diploids and corresponding tetraploids are represented by nine pairs of values from the trials in 1946 to 1949 (indirect comparison, diploids and tetraploids in different fields) and further by one pair of values from 1944 (isolation groups of diploid and tetraploid Steel-rye). The difference between the two average values 76,50 and 59,50 is obviously significant, and many other data also demonstrate that seed setting in diploid rye is, indeed, higher than in tetraploid rye. The value of the tetraploids, 59,50, represents 78 per cent of the value of the corresponding diploids and, thus, seed setting in autotetraploid rye is about 22 per cent lower than in diploid rye. The other tetraploid categories had slightly higher values, corresponding to 80 and 81 per cent of the seed setting of the diploid material. These figures, of course, are far from exact, but they strongly indicate that seed setting in tetraploid rye is not reduced with more than a quarter in comparison to diploid rye.

B. POLLEN FERTILITY.

Pollen fertility in autotetraploid rye is apparently excellent, and plants with reduced fertility, which are remarkably frequent in ordinary rye (*cf.* MÜNTZING, 1939, 1946), seem to be more rare in tetraploid rye. In 1938 some pollen samples of the tetraploid American rye (Rosen-rye) as well as of the corresponding diploid were examined, and in 1941 and 1942 new counts were made in the tetraploid strain. The following results were obtained:

	Per cent good pollen									
	30 - 40 - 50 -			60 -	70 -	80 -	90 -	100	n	M
1938, diploid				1	1	—	3	6	11	85,9
» , tetraploid						1	3	8	12	90,8
1941, »							4	26	30	93,7
1942, »	1	2	—	4	10	38	157		212[1]	90,9

[1] In addition to these 212 plants there was one individual with non-dehiscing anthers.

Though rather fragmentary, these data are nevertheless sufficient to show that the great majority of the tetraploid plants have 90—100 per cent apparently good pollen grains, and that the reduction of fertility on the female side is not accompanied by a corresponding decrease in the percentage of morphologically good pollen grains. Whether all these pollen grains are also really functional is not known.

No systematic studies of pollen fertility have been carried out in the other tetraploid rye varieties but, *e. g.*, in Steel-rye several pollen samples have been observed and found to be quite good.

C. INCOMPATIBILITY BETWEEN DIPLOIDS AND TETRAPLOIDS.

As is often the case in autotetraploids, there is also in rye a pronounced barrier of incompatibility between the diploid original forms and the new strains with doubled chromosome number. This barrier, however, is not absolute, and it is possible, though with difficulty, to raise triploid hybrids from both reciprocal combinations.

In 1944 258 flowers of tetraploid rye were pollinated with the pollen of diploid rye; this resulted in only 5 seeds, corresponding to a percentage of seed setting of 0,02. In the reciprocal combination 268 flowers were pollinated, but not a single seed was obtained. In crosses between two diploid strains of rye 330 seeds were obtained from 994 flowers pollinated. This corresponds to a seed setting percentage of 33,20. Unfortunately no crosses between tetraploids were carried out this year.

The whole problem of incompatibility between diploid and tetraploid rye was then left to one of my students, Mr. S. ELLERSTRÖM. In a recent paper HÅKANSSON and ELLERSTRÖM (1950) describe the embryological features in reciprocal crosses between diploid and tetraploid rye as well as in the control crosses, diploid × diploid and tetraploid × tetraploid. In this connection I just want to cite ELLERSTRÖM's data on the results of his crosses (*l. c.*, p. 263). In $2n \times 4n$ he obtained 36 kernels from 1275 pollinated flowers. 17 of them germinated and 4

plants were triploids, the other diploids, probably resulting from contamination with haploid pollen. In the reciprocal cross, $4n \times 2n$, 20 seeds were obtained from 783 pollinated flowers. Six of them germinated and four triploid plants were obtained, the other two plants being tetraploid.

The low seed setting in these crosses is not due to defective pollen tube growth and a reduced degree of fertilization. Fertilization occurs readily in $2n \times 4n$ as well as $4n \times 2n$, but later on specific irregularities in the endosperm development occur, which are somewhat different in the reciprocal crosses, but which in both combinations lead to the death of most of the embryos (HÅKANSSON and ELLERSTRÖM, *l. c.*).

Under such circumstances it is easy to understand that seed setting in tetraploid rye is much reduced if it is exposed to the pollen of ordinary rye. Many triploid zygotes are then formed, most of which fail to develop into mature seeds. It is still uncertain whether and to what extent seed setting is reduced in open-pollinated diploid rye, also exposed to the pollen of tetraploid rye. Preliminary evidence indicates that in such cases none or few triploid aborting seeds are formed, and it is rather probable that when diploid rye plants receive haploid as well as diploid pollen grains, the haploid ones will be more effective in fertilization. This, however, remains to be more closely analysed, especially by making pollinations with a mixture of haploid and diploid grains. Preliminary results of such pollinations are discussed below (p. 78).

4. PHYSIOLOGICAL AND CHEMICAL CHARACTERS.

Under this heading may be given various data on sprouting ability, winter hardiness, earliness and baking properties.

A. SPROUTING ABILITY.

The big kernel of tetraploid rye seems to give the young seedling a better start than the smaller kernel of ordinary rye. At any rate the percentages of seedlings per seeds sown have uniformly been higher in the tetraploids. With regard to Steel-rye, data are available from six years, in which diploids and tetraploids were sown in the same trial. The size of the plots and the number of replications varied somewhat in the different cases but, anyhow, in every case diploids and tetraploids were sown simultaneously, close to each other and with some replications. The average values obtained were the following:

Year	Diploid	Tetraploid	Tetraploid in per cent of diploid
1942	73,0	85,0	116,4
1943	75,0	87,0	116,0
1944, trial A	84,5	91,7	108,5
» , » B	78,2	85,5	109,3
1946, » A	71,0	82,0	115,5
» , » B	66,9	79,8	119,3
1949	86,8	92,8	106,9
1950	77,0	79,1	102,7

According to these values, the superiority of the tetraploid ranged from 2,7 to 19,3 per cent in the different years and trials, the general average value being 11,8.

In this autumn (1950) diploid and tetraploid King-rye could also be directly compared, 4 plots of each kind alternating with each other. The average per cent value of the diploid was 65,5 and of the tetraploid 83,0, the latter value being 26,7 per cent higher than the first one. This difference between 65,5 and 83,0 is significant, an analysis of variance giving a v^2 of 17,58 and a P value intermediate between 0,01 and 0,001.

Now, of course, germination and sprouting ability are highly influenced by the way in which the seed cultures have been treated, the conditions during the harvest, *etc.*, and in this respect the tetraploids and diploids are not strictly comparable. In most cases the tetraploid seed material was harvested from our own isolation groups, whereas the diploid rye sown was derived from larger propagations. However, this diploid seed material has been obtained from the Swedish Seed Association or the Swedish Seed Company and has been controlled to have good germination ability and to be normal also in other respects. Thus, there is scarcely any reason to assume that the tetraploid seed material should have been more favoured than the diploid one. At any rate, this is certainly not the case in the two last years, in which the kernels of tetraploid rye were obtained from large propagations in which no selection had been undertaken. As also in these cases the tetraploids had higher values than the diploids, it is highly probable that the larger kernel of tetraploid rye is really favourable as regards the germination and the ability of the coleoptile to penetrate the soil.

From various trials during the years 1943—1950 data are also available concerning the sprouting ability of other tetraploid varieties than tetraploid Steel-rye. If tetraploid Steel is given the value 100,0, the relative values of these other tetraploids were found to vary in the following way:

	Relative values (tetraploid Steel = 100,0) 70 — 75 — 80 — 85 — 90 — 95 - 100 - 105	n	M
Tetr. American	1 3	4	91,3
» Swed. × Am.	1 — — — — 2 1	4	92,5
» Steel × Am.	1 1	2	100,0
» Wasa II	2 2 1	5	96,5
» Steel × Wasa II	1 1	2	100,0
» Toivo	1	1	97,5
» Sangaste	1	1	92,5
» Crossing-group	1 — 2	3	94,2

Six of the average values are lower than 100,0 and none is higher. Of the total number of variates (each one representing an average value of a special trial) 4 are higher than 100,0 and 18 are lower. Thus, it is quite clear that on an average the other tetraploid rye varieties have less good sprouting ability than tetraploid Steel-rye, and there is no evidence that any single of them should be better than Steel.

B. FROST RESISTANCE.

So far, only one direct freezing experiment has been carried out with diploid and tetraploid Steel-rye. It was performed by the staff of the Swedish Seed Association in the standardized way used for testing winterhardiness in winter wheat and other cereals. Five boxes of each category were sown with 90 kernels per box, the seedlings then being exposed to the three different temperatures — 14°, — 15° and — 16° C. The result of this experiment was the following:

	Temperature (C°)	Number of plants alive	dead	Not germinated
Dipl.	— 14	48	30	12
Tetrapl.	»	48	42	—
Dipl.	— 15	43	30	17
Tetrapl.	»	39	48	4
Dipl.	»	55	20	15
Tetrapl.	»	50	35	5
Dipl.	— 16	15	52	23
Tetrapl.	»	14	65	11
Dipl.	»	30	46	14
Tetrapl.	»	11	79	—

The absolute number of plants surviving — 14° and — 15° is about the same in the diploid and tetraploid category, whereas in one pair of boxes exposed to — 16° the tetraploids were less successful than the diploids. Considering that germination in the diploid boxes was less

good than in the tetraploid ones, the general conclusion is that in this particular experiment tetraploid Steel-rye was slightly less frost resistant than diploid Steel-rye. More experiments of this kind should be carried out in order to get more reliable data. However, other data, on a much larger scale, already demonstrate that frost resistance in tetraploid Steel-rye is quite good and with certainty better than the frost resistance of South Swedish winter wheat.

The winter of 1946—47 was very severe, and at Svalöf as well as several of the branch stations the cereal trials suffered under an unusually long winter without snow cover and with strong and persistent winds. At Skara this winter was followed by an exceptionally dry period and all this led to an almost complete extinction of the wheat standards used in the rye trials. At the time of harvest only a few wheat plants were left, whereas the rye material had survived better and formed closed, though meagre plots. The tetraploid material (tetr. Steel-rye and three other strains) survived just as well as the diploid one. At Svalöf a quite similar result was obtained. Two wheat standards, Skandia III and Ergo, were used. Of these Skandia III was completely killed and Ergo badly damaged, whereas the rye, tetraploid as well as diploid, survived fairly well.

At Linköping the severe winter also decreased the yield of the wheat standards Ergo and Skandia III considerably, the yields per hectare being as low as 1510 and 1230 kg., respectively, in the diploid trial, and 1400 and 1180, respectively, in the tetraploid trial. The rye material again survived better and gave about twice as high a yield as the wheat, the hectare values being 3050 for diploid Steel-rye and 3150 for tetraploid Steel-rye. This resulted in the relative yield values 225,0 for tetraploid Steel and 202,0 for diploid Steel. In the same way tetraploid Wasa II had the relative yield value 199,3, the corresponding value of diploid Wasa II being 180,8 (*cf.* Table 13).

Dr. WÅHLSTEDT, Head of Linköping branch station, has also given me the following figures, indicating the density of the stand in the autumn, spring and summer. This density was estimated by using a scale from 0 to 10, the latter value representing the maximum degree of density.

The values in brackets are relative values, obtained by giving the average of the two wheat standards the value 100,0. As usual, diploid and tetraploid rye varieties were grown in separate trials but with the same wheat varieties as standards. A comparison between the absolute and relative values from October and April demonstrates that the tetra-

	October 30th, 1946	April 21th, 1947	July 23rd, 1947
Dipl. Steel-rye	9,2 (104,5)	7,4 (192,2)	7,2 (257,1)
» Wasa II	9,1 (103,4)	7,3 (189,6)	7,3 (260,7)
Ergo (wheat stand.)	8,5	3,8	3,0
	(100,0)	(100,0)	(100,0)
Skandia III (wheat stand.)	9,1	3,9	2,6
Tetr. Steel-rye	9,8 (105,4)	7,2 (271,7)	7,3 (208,6)
» Wasa II	9,4 (101,1)	7,0 (264,1)	7,0 (200,0)
Ergo (wheat stand.)	8,9	2,2	3,5
	(100,0)	(100,0)	(100,0)
Skandia III (wheat stand.)	9,7	3,1	3,5

ploid rye varieties had survived the winter at least as well as the diploid ones.

In all these values several factors other than specific frost resistance are no doubt involved, but they certainly indicate quite strongly that frost resistance in tetraploid Steel-rye as well as in the other tetraploid rye varieties is by no means inferior. Tetraploid rye is able to stand extreme winter conditions just as well as diploid rye. This is at any rate true of the tetraplod varieties Steel-rye and Wasa II.

C. EARLINESS.

Unlike most other autotetraploids, tetradpoid rye shows no retardation in speed of growth and reaches the flowering stage and maturity at the same time as diploid rye. From a practical point of view it is of course important that there is no significant decrease in earliness in the tetraploid.

In the first place this was observed directly in the field trials before 1946, in which diploids and tetraploids were grown side by side. It was also observed in smaller observation plots of diploid and tetraploid Steel-rye, which were grown more recently, the material being replicated several times with diploid and tetraploid plots alternating. From these direct observations it may be concluded that, at least in Steel-rye, flowering in diploids and tetraploids generally starts in the same day. In some cases the tetraploid seemed to be half a day or one day later than the diploid, but no significant difference has so far been established neither with regard to the start of the flowering nor the time of maturity.

Several data are available concerning earliness in such trials in which diploids and tetraploids were grown in different fields. Accurate observations of this kind were, *e. g.*, made at the branch stations of the

Swedish Seed Association in the years 1948 and 1949. Among other things the total time in days from sowing in the autumn to maturity in the following summer was registered. The absolute values obtained were the following:

Number of days from sowing to maturity

	315	320	325	330	335	340	345	350	355	n	M
Dipl. Steel-rye	1	1	3	—	—	2				7	329,7
Tetr. »	2	—	2	1	2					7	328,2
Dipl. Wasa II	1	2	2	—	1	1				7	328,2
Tetr. »	2	1	1	1	2					7	327,5
» crossing-group ...	2	—	2	1	2					7	328,2
Ergo (wheat stand.)	1	2	2	3	—	3	1	1		13	334,0
Gluten »	1	1	—	—	—	2				4	331,2
Odin »			2							2	327,50

In most cases Ergo wheat was used as a standard but in some trials it was replaced by the varieties Gluten or Odin.

The data given above demonstrate that there is no significant difference in time of development between the diploid varieties Steel and Wasa II and their corresponding tetraploids, the average values of the latter ones even being slightly smaller than those of the diploids. This, however, is a very rough comparison, and more accurate results were obtained by a calculation of the relative values, giving the wheat standard in each trial the value 100,0.

This calculation resulted in the following figures:

Relative earliness (wheat stand. = 100,0)

	97,0—	98,0 — 99,0	100,0—101,0	n	M
Diploid Steel-rye	2	2	2	1	7	98,79
Tetraploid »	2	1	3	1	7	98,93
Diploid Wasa II	3	3	1		7	98,21
Tetraploid »	3	—	4		7	98,64
» crossing-group	3	—	3	1	7	98,79

Evidently there is not a single significant difference between any of these values and, thus, at least in Steel-rye and Wasa II the tetraploids are not later than the diploids. Also the crossing-group is just as early, and as this group is derived from a mixture of several different varieties and no selection for increased earliness has been undertaken it is obvious that earliness is no problem in tetraploid rye.

D. BAKING PROPERTIES.

Thanks to Professor ÅKE ÅKERMAN and Dr. JOHAN LINDBERG of the Swedish Seed Association preliminary baking tests and cereal-chemical analyses with tetraploid rye were carried out with material harvested in 1944 and 1946. From the detailed reports obtained I shall quote a few figures.

In 1944 samples of 1,8 kg. of diploid and tetraploid Steel-rye were ground to 0,9 kg. of flour. The raw protein content of the kernels was 11,2 per cent in the tetraploid and 10,3 per cent in the diploid, the corresponding figures of the flour being 5,8 and 5,5 per cent. Baking tests with this flour gave the following result:

	Grams of dough from 100 gr. of flour	Gr. of bread from 100 gr. of flour	Volume of the bread (in cm^3) from 100 gr. of flour
Tetraploid a	156	148	282
» b	156	145	279
Diploid a	155	140	250
» b	155	139	260

Both tetraploid samples gave a slightly heavier and clearly larger bread than the two diploid samples. On an average bread volume in tetraploid Steel-rye was 10,0 per cent larger than that of diploid Steel-rye. The appearance of the exterior and interior of the bread is shown in Fig. 17. The tetraploid bread was not only larger, it had also a better surface, better interior structure and better colour than the diploid bread. In the first place these characters are certainly correlated with the higher protein content in the tetraploid flour. Further evidence that tetraploidy in rye leads to a slight but significant increase in protein content was obtained in 1946. A cereal analysis of the kernels gave the following result:

		In dry matter			
	Water content %	Raw protein %	Crude matter %	Ashes %	Diastatic condition (ME)
Dipl. Steel-rye	13,6	11,0	2,1	1,8	28
Tetr. »	13,4	13,5	2,2	2,0	31
» Wasa II	13,7	12,4	2,5	2,1	28

That the difference in protein content between diploid and tetraploid Steel-rye is really significant is evident from a detailed analysis of the flour of these samples, this analysis being performed by the Chemical Department of the Seed Association under the guidance of Dr. LINDBERG.

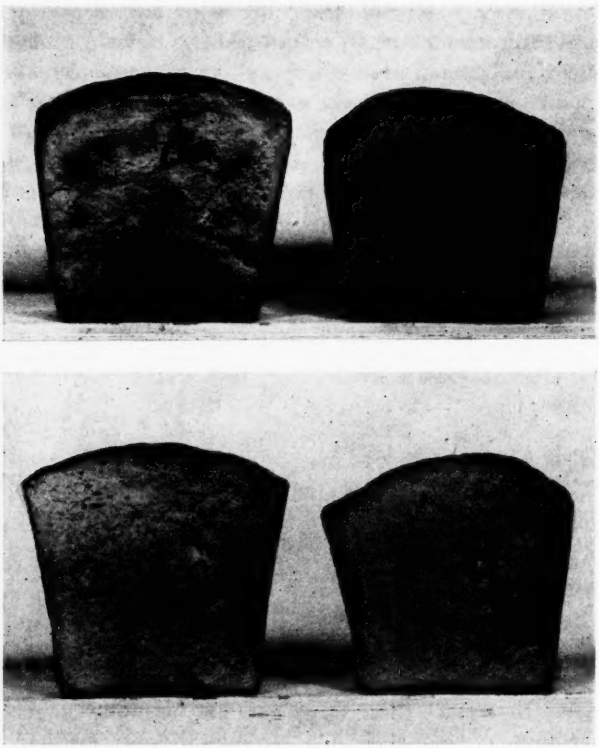


Fig. 17. Breads of diploid Steel-rye (to the right) and tetraploid Steel-rye (to the left). Outer surface above, inner surface of the same breads below.

The flour was fractionated in different ways, using different degrees of grinding, *etc.*, and this resulted in 13 different values of each sample. Without going into further details, which must be left to the specialists, I shall only quote the values on protein content in these samples:

Diploid Steel: 4,8—6,0—7,3—11,1—10,8—11,8— 9,8— 9,5—12,5—15,9—15,3—14,6—14,3
Tetrapl. » : 5,5—7,2—9,8—11,9—14,5—15,5—13,3—13,0—13,6—17,0—17,2—16,8—17,3
» Wasa: 5,3—6,7—9,0—12,2—13,6—13,8—11,8—11,6—13,5—16,7—16,7—14,2—16,3

The values above each other are comparable and, thus, it can be seen that every value in tetraploid Steel is higher than the corresponding one in diploid Steel. Unfortunately, diploid Wasa II was not available

for analysis, but the values of tetraploid Wasa are more similar to those of tetraploid Steel than to those of diploid Steel.

Cereal analyses and baking tests with tetraploid rye were carried out in the autumn of 1948 at the Cereal Laboratory of Saltsjökvarn, a big mill in Stockholm. The samples analysed were tetraploid Steel-rye from two different propagations (Flahult in the province of Småland and Ahleborg in Scania). Detailed reports were given concerning the results of the analysis together with the general statement that »the baking property of the tetraploid rye is better than in the samples of ordinary rye obtained this autumn».

More recently baking tests have been carried out also at other mills and factories and especially at the »Wasa Spisbrödsfabrik» at Filipstad, province of Värmland. This is a big factory making the typical hard rye bread, »knäckebröd», widely used in Sweden. I have been informed that for making this hard rye bread a high protein content is especially desirable, that the larger kernels of ordinary rye have a higher protein content than the smaller ones, and that the preliminary baking results with tetraploid rye are quite promising. If the protein content is significantly correlated to kernel size, this is, indeed, not surprising as the weight of the tetraploid kernel is about 53 per cent larger than that of the diploid kernel (*cf.* above p. 45). Unfortunately, I have not yet received any figures concerning these baking tests, but we have been informed that we are on the »right track» as regards the suitability of tetraploid rye for the production of the hard rye bread.

Thus, though more data concerning these large scale baking tests are urgently needed, the evidence and information already gathered strongly indicate that the baking properties of tetraploid rye are superior to those of ordinary rye both with regard to the ordinary soft bread and to the hard »knäckebröd».

5. YIELD.

A. KERNEL YIELD.

The first observations of kernel yield in rye were made in the American tetraploid. As this variety happened to be especially poor under Swedish conditions, the impression was obtained that induction of tetraploidy in rye would probably never lead to products of practical importance. The next strain produced, »Swedish × American», was somewhat more promising, having a much higher percentage of seed setting than the American tetraploid. However, it turned out to have a

poor tillering capacity and poor strawstiffness and was finally discarded. Much better results were obtained with the colchicine-induced tetraploid Steel-rye and also tetraploid Wasa II was rather satisfactory. Yield trials with these varieties, as well as with a hybrid population of the cross Steel × American, were undertaken in 1943 to 1945, growing diploids and tetraploids side by side. In 1943 and 1944 these trials gave the following relative yield values (diploid Steel = 100,0):

	1943	1944
Diploid Steel	100,0	100,0
Tetrapl. »	68,8	89,4
» Swed. × Am.	28,6	56,3
» American	24,5	42,7

In 1943 there were 3 replications and one kernel per hole (marker boards), in 1944 4 replications and 2 kernels per hole.

In 1945 the main trial was again sown with marker boards, and 2 kernels per hole, using rather big plots (2400 kernels) and 3 replications. The relative values obtained were as follows:

	All three replications		Second and third repl.	
	Absolute (gr.)	Relative	Absolute (gr.)	Relative
Diploid Steel-rye	2548	100,0	2220	100,0
Tetrapl. »	2045	80,3	2112	95,1
» American	1418	55,7	1383	62,3
» Steel × Am.	1922	75,4	2045	92,1
» Swed. × Am.	1632	64,1	1753	79,0
» Wasa II	2075	81,4	2167	97,6

According to the first series of values, based on all three replications, tetraploid Steel and Wasa II had about 20 per cent lower yield than diploid Steel-rye, the other tetraploids being still more inferior. It was observed, however, that in the second and third replications seed setting was on an average clearly better in the tetraploids and less good in diploid Steel-rye. At least with regard to the tetraploids the reason was later on found out to be that the plots of the first replication were grown close to another field trial with diploid rye, whereas the other replications were more protected from the pollen of the diploids. At the same time it was observed that seed setting of the tetraploids in the trial was less good than in the isolation groups. In the same year a field trial in Ugerup, where narrow plots of tetraploid Steel-rye were heavily exposed to ordinary rye pollen, gave a very poor seed setting and low yield.

TABLE 13. *Yield values of tetraploid and diploid Steel-rye.*

Year	Place of trial	Tetraploid		Diploid		Tetrapl./Diploid	
		Kg. per hectare	Relative weight	Kg. per hectare	Relative weight	Absolute	Relative
1946	Svalöf	4922	128,7	4658	95,7	1,06	1,34
1947	»	2151	142,9	2305	135,1	0,93	1,06
1947	Linköping	3150	225,0	3050	202,0	1,03	1,11
1947	Skara	1356	—[1]	1111	—[1]	1,22	—[1]
1947	Kalmar	3678	97,1	2373	157,0	1,55	0,62
1948	Svalöf	4614	119,7	5860	113,5	0,79	1,05
1948	Ultuna	4470	113,9	4940	135,0	0,90	0,84
1948	Skara	5820	147,3	4260	130,3	1,37	1,13
1948	Kalmar	6155	129,8	4765	120,2	1,29	1,08
1949	Svalöf	3525	110,7	3100	93,0	1,14	1,19
1949	Ultuna	4400	73,8	5015	82,7	0,88	0,89
1949	Linköping	5320	85,5	5560	102,0	0,96	0,84
1949	Skara	4270	106,6	5600	93,9	0,76	1,14
1949	Kalmar	3805	82,5	6385	119,9	0,60	0,69
1949	Norsbron	3798	264,7	6753	169,0	0,56	1,57
1950	Svalöf	4502	102,4	4623	91,3	0,97	1,12
1950	Skara	3540	98,7	6110	117,8	0,58	0,84
1950	Kalmar	4045	145,8	4950	146,4	0,82	1,00
1946—1950	Average	4085	—	4523	—	0,97	1,03

[1] Wheat standards destroyed by frost and drought.

As explained above (p. 57), all this means that triploid aborting zygotes are frequently formed when diploid and tetraploid rye are grown together. This especially tends to decrease the yield of the tetraploids and, thus, it was found to be necessary to study diploids and tetraploids in separate trials, using common wheat standards as a basis for an indirect comparison.

Since 1946 trials of this kind were undertaken every year at Svalöf and since 1947 also at some or all of the five branch stations of the Swedish Seed Association in South and Central Sweden (Linköping, Skara, Kalmar, Ultuna and Norsbron). A detailed discussion of all these trials would require too much space and, thus, the report will mainly be restricted to a discussion of the following three tables. Table 13 contains the main data so far obtained from all indirect comparisons of diploid and tetraploid Steel-rye. Reports of the results gathered in 1950 at the branch stations are not yet complete, and at the time of writing only those from Skara and Kalmar are available.

As the size of the plots has been different in different places and different years (generally ranging between 10 and 25 square meters), the absolute yield values are omitted and the calculated kernel yield in kilogram per hectare used instead. Still more important, however, are the relative values obtained by means of one or two wheat varieties present in the diploid as well as the tetraploid rye trials. The yields per hectare of the wheat standards are not given but may be computed from the other values.

In tetraploid Steel-rye the yield values per hectare in the 18 trials ranged from 1356 kg. (Skara 1947) to 6155 kg. (Kalmar 1948) with an average of 4085. In the first place the variation in yield is caused by marked differences in the severeness of the winters and other climatic conditions. 1947 was a very trying year not only at Skara but also at the other places of trial.

In diploid Steel-rye the corresponding variation in yield per hectare was from 1111 kg. (Skara 1947) to 6753 kg. (Norsbron 1949) with an average of 4523. The diploid and tetraploid series of hectare yield values were compared with an analysis of variance. The difference between the means is not significant, v^2 being smaller than 1,0 and P larger than 0,2.

The indirect comparisons with the help of the wheat standards is not easy as is evident from the very marked variation in the relative weight values. In the tetraploid series these values range from 73,8 to 264,7 and in the diploid series from 82,7 to 202,0. In the trial at Skara 1947 no relative values could be obtained, the wheat standards in both trials being destroyed by severe frost and drought.

The last two columns to the right in Table 13 represent the quotients obtained by dividing the hectare yields and relative yields of the tetraploid with the corresponding values of the diploid. Thus, values higher than 1,00 indicate that the tetraploid has been superior.

As regards the »absolute» values, *i. e.* the quotient between the hectare values, 7 values are higher than 1,00 and 11 are lower, the average of this series being 0,97. In the »relative» series, finally, 10 values are higher than 1,00, 6 values are lower and one value is exactly 1,00. The average of the whole series is 1,03. The absolute series evidently indicates a slight inferiority of the tetraploid, the relative series a slight superiority. The only sound conclusion that can be drawn is that in these 18 trials, during the five year period 1946 to 1950, diploid and tetraploid Steel-rye have been equal in average yield.

A quite similar survey of the results of trials with diploid and tetra-

TABLE 14. *Yield values of tetraploid and diploid Wasa II.*

Year	Place of trial	Tetraploid		Diploid		Tetrapl./Diploid	
		Kg. per hectare	Relative weight	Kg. per hectare	Relative weight	Absolute	Relative
1946	Svalöf	4433	115,9	4316	88,6	1,03	1,31
1947	»	2011	131,2	2147	125,9	0,94	1,04
1947	Linköping	2790	199,3	2730	180,8	1,02	1,10
1947	Skara	1422	—[1]	1222	—[1]	1,16	—[1]
1947	Kalmar	3106	82,0	2367	156,6	1,31	0,52
1948	Svalöf	4000	103,7	6391	113,5	0,63	0,91
1948	Ultuna	3805	96,9	5150	140,7	0,74	0,69
1948	Skara	5720	144,8	4190	128,1	1,37	1,13
1948	Kalmar	5240	110,5	4490	113,2	1,17	0,98
1949	Svalöf	3225	100,0	3116	93,5	1,03	1,07
1949	Linköping	5240	84,2	4690	86,1	1,12	0,98
1949	Skara	3920	97,9	5010	84,0	0,78	1,17
1949	Kalmar	3480	72,6	5860	110,0	0,59	0,66
1949	Norsbron	3278	228,4	6102	152,7	0,54	1,50
1946—1949	Average	3691	—	4127	—	0,96	1,00

[1] Wheat standards destroyed by frost and drought.

ploid Wasa II is given in Table 14. Data on this material are available from 14 trials. Passing directly to the comparison between tetraploid and diploid in the right part of the table, we find that the quotient between the hectare yields is larger than 1,0 in 8 cases and lower in 6 cases, the average value being 0,96. As regards the quotient between the relative values, this quotient is higher than 1,0 in 7 cases and lower in 6 cases. Thus, neither in Wasa II is there any significant difference in kernel yield between the diploid and tetraploid strains.

Finally, it remains to compare the yields of different tetraploid varieties, yield data being available not only for tetraploid Steel and Wasa II but also for the tetraploid crossing-group and five other strains. The simplest way to perform this comparison is to consider tetraploid Steel as a standard with the yield value 100,0 and to express the yields of the other strains in per cent of this value. The result of this calculation is given in Table 15.

Tetraploid Wasa II has been compared with tetraploid Steel in 19 different trials, and without exception the yield has been lower. The total average is 87,77, the difference between the varieties thus being about 12 per cent. Tetraploid Steel × Wasa II has been compared with

TABLE 15. *Relative yield in different tetraploid varieties.*

Tetraploid varieties	Relative kernel yield (tetrapl. Steel = 100,00)																Number of trials	Average
	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125		
Wasa II						1	—	4	7	6	1						19	87,77
Steel × Wasa II								2	1	—	3	1					7	92,50
Crossing-group								1	1	6	6	1	—	—	—	1	16	95,94
American						1											1	72,50
Swed. × Am.						1											1	72,50
Steel × Am.								1									1	82,50
Sangaste					1												1	67,50
Toivo	1	—	1														2	57,50

tetraploid Steel in 7 trials, the average relative value in this case being 92,50. This value is evidently just intermediate between the values of the parent strains, 100,0 and 87,77.

The tetraploid crossing-group is rather high-yielding, the average relative value from 16 trials being 95,94. It is very close to tetraploid Steel-rye, but the difference of 4 per cent is certainly significant, 14 of the 16 values being lower than 100,0. Of the other varieties only one or two values are available, but these values are all quite low and demonstrate that tetraploid Steel-rye is superior to all these varieties. All data in Table 15 are derived from trials in which no diploids but only different tetraploid strains and wheat standards were present. It may to some extent be supplemented by the data from 1943 to 1945 given above (p. 66), in which some other tetraploid strains were grown together with tetraploid Steel-rye. Though the yield values were all disturbed by the presence of diploids in the same trials it is quite certain that at least tetraploid American and Swedish × American are inferior to tetraploid Steel.

Thus, considering all data available, it is quite certain that tetraploid Steel-rye is the most high-yielding strain in my present collection of tetraploid rye types.

B. STRAW YIELD.

In Sweden the straw yield is of no practical importance but from a theoretical point of view it may be of interest to know, how the tetraploids compare to the diploids in this respect, the straw yield evidently being a good indication of the degree of vegetative development. As usual, however, comparison is difficult on account of the necessary

separation of diploids and tetraploids to different fields. In 1948 and 1949 the following data were obtained from the branch stations of the Seed Association:

	Straw weight (kg. per hectare)									
	4000—5000	— 6000	— 7000	— 8000	— 9000	— 10000	— 11000	n	M
Dipl. Steel			3	2	1			6	7167
Tetr. »			2	2	—	—	2	6	8167
Dipl. Wasa II			3	1	1	2		7	7786
Tetr. »	1	2	2	—	1	1		7	6643
» crossing-group		1	3	1	—	1	1	7	7500

The table seems to indicate that straw weight in tetraploid Steel is greater than in diploid Steel, while in Wasa II the tetraploids are inferior. However, a statistical test showed that these differences are not significant. The relative weight values were also calculated with the use of the wheat standards. This gave the figures 98,3 (dipl. Steel), 120,0 (tetr. Steel), 97,9 (dipl. Wasa II), 100,7 (tetr. Wasa II), 115,0 (tetr. crossing-group). Neither in this case are the differences significant. Thus, at present it can only be stated that no significant differences in straw weight have been found between diploids and tetraploids, though a superiority in this respect for tetraploid Steel-rye is indicated.

IV. DISCUSSION.

Cytologically, tetraploid rye behaves like a true autotetraploid, forming a high frequency of quadrivalents at meiosis. In the four varieties examined the average number of quadrivalents at first metaphase ranged from 3,95 to 4,29, the differences between these values not being significant. This is of interest, because in one of the varieties, tetraploid Steel-rye, rigid selection for increased fertility had been performed during 7 years, whereas two of the other varieties, tetraploid Sangaste and Toivo, were of recent origin and had not been subject to selection. Thus, it is rather clear that in the present case selection for increased fertility had little or no effect on the mode of chromosome pairing at meiosis.

Meiosis in tetraploid rye was previously studied by MÜNTZING and PRAKKEN (1941), CHIN (1943) and O'MARA (1943). In the first of these cases the material consisted of tetraploid spikes of a spontaneous diploid-tetraploid chimaera. The average number of quadrivalents was 2,33 and, thus, considerably lower than in the present material. In a tetraploid

strain of summer rye produced by LI, CHIN found an average of only 1,05 quadrivalents per cell. Differences in frequency between strains may of course occur, but another possible explanation is that the number will tend to be lower, if fixation is poor and only a small proportion of the metaphase groups can be analysed. Environmental conditions may also be of importance.

The present data confirm previous observations by the authors mentioned that univalents often occur at first metaphase, divide at first anaphase and lag at second anaphase, this lagging often giving rise to micronuclei, which may be counted in the tetrads. At I-A the regular distribution, 14—14, is the most frequent one, but not very predominating. In the present material 38 per cent of the cells showed other types of distribution, which may lead to a rather high proportion of aneuploid gametes.

The average frequencies of univalents at I-M ranged from 0,23 to 0,34 per cell and are lower than in the material studied by O'MARA, his average being 0,68 for euploid plants with 28 chromosomes. This material was a strain of tetraploid summer rye produced with colchicine by SAX. Special attention was paid by O'MARA to a rather frequent occurrence of different types of bridges at I-A, some of them demonstrating a structural differentiation between the conjugating chromosomes. Such bridges were occasionally seen in the present material (*cf.* Fig. 10) but in a quite low frequency. A very interesting phenomenon observed by O'MARA is the occurrence of single pollen mother cells or groups of p.m.c. deficient for a variable number of chromosomes. Such apparent »somatic reduction», first observed by LOVE (1938) in wheat hybrids and later on found in several other cases, does not seem to occur in my present material of tetraploid rye. Thus, regarding frequency of univalents and anaphase bridges as well as the occurrence of cells with reduced chromosome number, the summer rye studied by O'MARA clearly differs from my material of tetraploid winter rye.

The mode of meiosis must lead to the formation of aneuploids in the offspring, and in my material 22,47 per cent of the 808 plants examined had 26, 27, 29 or 30 chromosomes instead of 28. The most frequent class of aneuploids is represented by the plants with 29 chromosomes, and hyperploids in general were found to be significantly more frequent than hypoploids. In the variety Wasa II hypoploids were even entirely absent. A similar result was previously obtained in autotetraploid *Galeopsis pubescens*. In this species hypertetraploids were found

to be more than four times as frequent as hypotetraploids (MÜNTZING, 1941).

The precise effects of aneuploidy in tetraploid rye may be considered in another paper. Undoubtedly, however, the occurrence of as much as 20 or 25 per cent of aneuploids will have markedly deleterious effects with regard to various properties of practical importance. In tetraploid Steel-rye, for instance, the degree of tillering is about 12 per cent lower than in diploid Steel-rye. Part of this difference, at least, may be due to a weaker development or death of the aneuploids.

The presence of aneuploids will certainly also reduce the average fertility and yield to some extent. A possible way of diminishing the bad effects of aneuploidy is to sift the seeds before sowing and remove the smallest fraction. As demonstrated previously (MÜNTZING, 1943), the aneuploid seeds, and especially the hypotetraploid ones, are on an average less well developed and more shrivelled than the euploid ones. A special trial was sown this autumn, in which plots from sifted and non-sifted seeds of the same variety will be compared.

It should be observed, however, that only a quite small proportion of the seeds of tetraploid rye are obviously shrivelled. On an average, kernel quality in tetraploid rye is surprisingly good and not inferior to the quality in ordinary rye. Thus, the lower hectolitre weight (p. 47) is not an expression of poor quality but only a consequence of the large size of the tetraploid kernel.

The aneuploids in tetraploid rye are not conspicuous and cannot be distinguished at all under ordinary field conditions, when the seeds have been sown with machine. A field of tetraploid rye looks just as uniform as a field of diploid rye and, as far as our experience goes, it is just as constant. At any rate, possible reversions from tetraploidy to diploidy must be very rare and have not been observed. This conclusion is further supported by the fact that a population of tetraploid Steel-rye has been grown at the agricultural station Flahult in 4 years and has remained constant and typical without selection. However, special care must of course be taken to avoid intermixture or crossing with diploid rye. As described previously (MÜNTZING, 1943), crosses with diploid rye may lead to apparent reversions to diploidy.

Morphologically, tetraploid rye differs from diploid rye in the way which is generally characteristic of autotetraploids. MÜNTZING (1936, pp. 282—283) studied these differences and found them to be the following: (1) The stem of the tetraploid is thicker and stouter than that of the diploid. (2) The tetraploid is taller than the diploid. (3) The tetra-

ploid has larger leaves than the diploid. (4) The tetraploid has thicker leaves than the diploid. (5) The leaves are relatively shorter and broader in the tetraploid. (6) The leaves are of a darker green in the tetraploid. (7) The flowers and floral parts are bigger in the tetraploid. (8) The seeds are bigger in the tetraploids.

Measurements undertaken prove that points 2, 7 and 8 apply to the rye material. To this may be added point 1, at least with regard to the thickness of the stem, as well as points 4 and 6. No exact measurements of the leaves have yet been undertaken, but from observations alone it is certain that the leaves are larger (point 3), at least in the autumn, and probable that their leaf index is changed in the way indicated by point 5. CHIN (1943) says that his tetraploids are taller, the ears longer, flowers, seeds, pollen grains and stomata larger. SENGBUSCH (1940, 1941), working with colchicine-induced tetraploid winter rye of the variety Petkus, also mentions that the pollen grains of the tetraploid are larger, and the tetraploids characterized by a more vigorous development and a darker green colour than the corresponding diploids.

Seed setting in my material of tetraploid rye is surprisingly good, most of the present strains having an average of about 60 per cent or slightly more. Only the American tetraploid was clearly different, having a much lower value. SENGBUSCH (1941) reports that in an isolation group of his tetraploid winter rye the average per cent of seed setting was 40, the corresponding value in a tetraploid strain of summer rye being 47,6. CHIN (1943) found only 23,8 per cent of seed setting in his tetraploid summer rye, the corresponding value of the diploid strain being 75 per cent. It is not stated, however, whether the tetraploid material was isolated from the pollen of diploid rye.

The percentage of seed setting in diploid rye is significantly different in different varieties, and much selection work has been carried out in order to increase the average seed setting. To a certain extent this work has been successful (*cf.* MÜNTZING, 1946) and, hence, selection of this kind should also be effective to a certain extent on the tetraploid level. Now, of course, this selection work will be difficult, if the diploid material, from which the tetraploid was produced, has already obtained a high degree of fertility. For that reason I am not sure whether the percentage of seed setting in tetraploid Steel-rye has really been improved by the nine years of selection after its production. This is difficult to know, as we cannot make direct comparisons between the original tetraploid material and the present tetraploid representatives of this variety. Comparisons with diploid Steel-rye are also of limited value,

as diploids and tetraploids are grown in different fields and the environmental conditions may affect the degree of seed setting rather markedly. Finally, there are obvious differences between years, certainly caused by the climatic conditions at the time of flowering. Thus, more data, covering a longer period of years than those given in Table 12, are needed before anything definite can be said about the possibilities of increasing the percentage of seed setting by selection. Owing to the high frequency of quadrivalents at meiosis, we can probably never expect to reach the same degree of seed setting in the tetraploids as in the diploids. Seed production and yield may certainly more easily be improved by selection for a higher number of tillers and other quantitative characters such as number of flowers per ear and kernel weight.

Anyhow, the average percentage of seed setting reached in tetraploid Steel-rye is relatively high and not lower than in some diploid rye varieties. To be more precise, the general average value of this tetraploid strains is now 62 per cent and in favourable years it may be considerably higher (73,2 per cent in the trial of 1947). Seed setting in the diploid Swedish land variety »Östgöta gråråg» was found to be as low as about 65 per cent (MÜNTZING, 1946). ULRICH (1902) found the average percentages of seed setting in the three German rye varieties Petkus, Probsteier and Schlanstedter to be 78, 61 and 79 per cent, respectively. LEITH and SHANDS (1938), working with American diploids, find a seed setting ranging from 60 to 80 per cent, and PETERSON (1934) states that in his material usually about one-third of the florets fail to set seed. According to my own observations, diploid Sangaste has also a relatively poor seed setting, whereas diploid Wasa II and especially diploid Steel are better in this respect. In Table 12 five values correspond to trials or isolation groups in which diploid Steel-rye was separated from tetraploid Steel-rye. The average of these values is 78,5. Another material of diploid Steel-rye was studied by me in 1937—1939 and found to have an average seed setting of about 80 per cent (MÜNTZING, 1946). The average value of tetraploid Steel-rye (62) in per cent of the two values of diploid Steel-rye just mentioned will be 80,3 and 78,8, respectively. Thus, we may conclude that seed setting in tetraploid Steel-rye is about 20 or possibly 25 per cent lower than in diploid Steel-rye.

What is the cause of this decrease? This question is partly answered in a recent paper by HÅKANSSON and ELLERSTRÖM (1950), dealing with the embryology of my tetraploid Steel-rye as well as with the results of reciprocal crosses between diploid and tetraploid rye. As a rule, embryo

sac development is normal in tetraploid Steel-rye, and degenerating embryo sac mother cells or macrospores are not more frequent than in diploid rye. The only indication of haplontic sterility on the female side is the occasional occurrence of an abnormal mode of embryo sac development. However, the reduced degree of seed setting is in the first place caused by two other phenomena, *viz.* non-fertilization of apparently normal embryo sacs and especially by zygotic disturbances during the seed development. The development of the endosperm, or sometimes of the embryo, may be abnormally slow and lead to abortion of the young seed. The effect of disturbed endosperm development at later stages may be limited to some degree of shrivelling. Similar disturbances may also occur in diploid rye, especially in inbred material (LANDES, 1939), but were found to be rare in diploid Steel-rye. Thus, the reduced seed setting in tetraploid rye is certainly due to lack of fertilization and to disturbed seed development. It is highly probable that in the first place these disturbances eliminate zygotes with gross deviations from the euploid number 28. The aneuploid aberrants are chiefly represented by plants with 29 or 27 chromosomes and more extreme deviations are rare. They are, indeed, more rare than should be expected from the rather irregular mode of meiosis, and, hence, zygotic selection is most probably at work. Whether the unfertilized embryo sacs really represent a random sample is still an open question, but from the descriptions given by HÅKANSSON and ELLERSTRÖM (*l. c.*) the non-fertilization scarcely seems to be selective and dependent on the chromosome number of the embryo sac.

Lack of adequate pollination may seem unlikely as the anthers in tetraploid rye are quite normal and shed large quantities of apparently good pollen grains. A possible reason for a lower degree of pollination in the tetraploid than in the diploid is the fact that the pollen grains of the tetraploid are larger and heavier and may be less easily carried by the wind. The reduced degree of tillering in the tetraploid may also to some extent tend to reduce the seed setting. The density of the stand is really of importance as demonstrated by observations in the American tetraploid (p. 53). In dense stands, obtained by sowing 2 kernels per marker board hole, seed setting was significantly higher than in less dense stands, obtained by sowing 1 kernel per hole.

The apparently perfect pollen fertility in tetraploid rye is remarkable and recalls a similar state of things in, *e. g.*, *Dactylis* (MÜNTZING, 1937). In this genus autopentaploids had excellent pollen fertility, whereas their triploid mother plants were completely male sterile. In

Poa alpina aneuploid and partially autopolyploid apomicts with 30—50 chromosomes also have very good pollen (MÜNTZING, 1932, 1940). Thus, in cases of typical or partial autopolyploidy the pollen seems to become very unsensitive to quantitative variation in chromosome number, and in rye the duplication of the chromosome number also seems to remove the partial sterility frequently occurring in diploid rye (MÜNTZING, 1939, 1946). LUNDQVIST (1947) finds that autotetraploid Steel-rye is more self-fertile than diploid Steel-rye and that it is more resistant to inbreeding degeneration.

Indications of an incompatibility barrier between diploid and tetraploid rye were first observed by SENGBUSCH (1940, 1941), who found that tetraploid rye exposed to the pollen of ordinary rye gave a much lower percentage of seed setting than isolation groups of the same tetraploid variety. These preliminary results were confirmed by our own observations and by crossing experiments, in which diploid and tetraploid rye were hybridized in both reciprocal combinations. Crosses with the tetraploid as female parent gave a seed setting percentage of 0,02, and in the reciprocal cross not a single viable seed was obtained from 268 flowers. In 1945 more extensive crossing experiments of the same type were carried out by my collaborator S. ELLERSTRÖM and in connection with this work embryological investigations were carried out by HÅKANSSON and ELLERSTRÖM (*l. c.*). The main result of this work is that the low seed setting in these crosses is not due to bad pollen tube growth and a reduced degree of fertilization. Fertilization occurs readily in $2n \times 4n$ as well as $4n \times 2n$, but later on specific irregularities in the endosperm development occur. They are somewhat different in the reciprocal crosses, but in both combinations they lead to the death of most of the embryos. Under such circumstances seed setting in tetraploid rye will be much reduced if it is exposed to the pollen of ordinary rye, many triploid zygotes being formed, most of which fail to develop into mature seeds. A quite similar situation prevails in tetraploid *Sinapis alba* exposed to pollen of diploid strains of the same species (OLSSON and RUFELT, 1948).

From a practical point of view it is necessary to know to what extent seed setting is reduced in open-pollinated diploid rye which is also exposed to the pollen of tetraploid rye. As pointed out above (p. 57), it is rather probable that the haploid pollen grains may be more effective in fertilization. Such is the case, *e. g.*, in maize (RANDOLPH, 1935), but in rye we don't have the long styles favouring the differentiation between the two groups of pollen tubes. Indeed,

HÅKANSSON and ELLERSTRÖM (*l. c.*) find that fertilization occurs readily in both the combinations $4n \times 2n$ and $2n \times 4n$. However, even a slight difference in rapidity may be sufficient to differentiate n and $2n$ pollen grains, if they are attached to the stigma simultaneously. Preliminary experiments with mixed pollinations indicate that such is really the case. Mr. ELLERSTRÖM has permitted me to quote the following result obtained by him in 1945. He pollinated 156 flowers in spikes of tetraploid rye with a pollen mixture consisting of 2 parts of haploid pollen (from diploid plants) and 1 part of diploid pollen (from tetraploid plants). Only 6 kernels were obtained, which corresponds to a seed setting percentage of 3,8. Five of these kernels could be brought to germination and gave two plants with $2n = 27$, one with $2n = 28$, one with $2n = 29$ and one plant which probably had $2n = 20$. At the same time 104 flowers of diploid spikes were pollinated with a mixture of 1 part of haploid pollen and 2 parts of diploid pollen. The result was 38 kernels, which corresponds to a seed setting of 36,5 per cent. Of these 38 kernels 35 germinated, and all of these plants were found to be diploid, having $2n = 14$.

These results clearly indicate that when the diploid is the female parent, the haploid pollen grains are more effective, and perhaps exclusively effective, in fertilization. When the tetraploid is the female parent, the haploid pollen tubes are again more effective in fertilization than the diploid ones and produce a high proportion of aborting triploid seeds. Only a small proportion of the seeds are viable, and these seeds are chiefly the result of fertilization by diploid pollen tubes. As this problem is of prime importance also from the practical point of view, more experiments of this kind should be performed. However, the evidence already obtained certainly indicates that when diploid and tetraploid rye are grown close to each other, the tetraploid will suffer more from the pollen mixture than the diploid.

Vigour and fertility are remarkably good in autotetraploid rye and better than in most induced autopolyploids. As a rule, doubling of the chromosome number also leads to changed physiological properties (*cf.* MÜNTZING, 1936, pp. 293—298), of which the most typical one is a decrease in the rate of development. The tetraploid rye scarcely shows any retardation, the only evidence in this direction being a slower rate of development of endosperm and embryo (HÅKANSSON and ELLERSTRÖM, 1950). Judging from the preliminary experiments with mixed pollinations, the pollen tubes of tetraploid rye may also be slightly slower than those of ordinary rye. However, the rate of plant development from germination to maturity is not reduced in tetraploid rye,

which is fortunate from a practical point of view. With regard to resistance to frost and drought, the tetraploids also seem to be equal to diploid rye, and as to the chemical properties, which are also frequently changed in autotetraploids, a difference in protein content of the kernel was established, the tetraploid being superior in this respect. This higher protein content is probably responsible for the better baking quality of tetraploid rye, the bread volume of tetraploid Steel-rye being about 11 per cent larger than in diploid Steel-rye. In autotetraploid maize the nitrogen content is also higher than in diploid maize (ELLIS, RANDOLPH and MATRONE, 1946).

The experience gathered so far concerning the practical value of tetraploid rye may be summarized as follows:

The best tetraploids strains are *equal* to the corresponding diploids with regard to yield, strawstiffness, earliness, frost resistance and drought resistance. The equality in yield is the outcome of a balance between two positive and four negative properties of the tetraploids. The positive ones are the big kernel which is about 53 per cent heavier than in diploid rye and the superior sprouting ability. The negative properties are: (a) A reduction of the percentage of seed setting with 20—25 per cent, (b) a reduction of the degree of tillering with about 12 per cent (in tetr. Steel-rye), (c) a lower number of flowers per spike, (d) a greater tendency to shed the basal spikelets before maturity.

The present tetraploids are *inferior* to diploid rye in the following respects: (a) The straw is too tall, making harvesting with a combiner difficult, (b) the big kernel size makes it necessary to use somewhat greater quantities of seeds for sowing, (c) tetraploid rye must be grown isolated from ordinary rye in order to avoid a reduction in seed setting.

With regard to the first point, it may be mentioned that a new tetraploid strain is already available (tetraploid King-rye), in which the straw is much shorter than in the other tetraploid varieties and, thus, by further breeding the disadvantage of the taller straw of tetraploid rye may certainly be diminished. With regard to the second point, it may be said that the superior sprouting ability of the tetraploids probably compensates part of the need for greater quantities of seed. A special field trial with different quantities of seed as well as with different quantities of nitrogen manuring was carried out 1949—1950 under the guidance of Doctors O. TEDIN and A. HAGBERG of the Swedish Seed Association. No differences obtained were significant, and in fact the highest yield was obtained from the plots representing the lowest quantity of seeds (200 kg. per hectare). More data are needed, but

already now it is rather clear that it is not necessary to compensate the 53 per cent larger 1000-grain weight of the tetraploid with 53 per cent more seed. Under such circumstances the difference in quantity of seed needed for diploid and tetraploid rye is only of minor importance. The same may now be said about the necessity of isolating tetraploid rye from diploid rye in order to prevent a decrease in seed setting and yield. The experience so far gathered (p. 52) shows that in large blocks of tetraploid rye the deleterious effect of ordinary rye pollen is very slight. Thus, though some degree of isolation is desirable it will not involve any real difficulty.

In two respects tetraploid rye is *superior* to ordinary rye, *viz.* with regard to sprouting ability and baking quality. These properties are probably both correlated with the increased size of the kernel. The big kernal produces a large and vigorous seedling, which has a greater ability to penetrate the soil than the weaker diploid seedling. The increase in kernel size also leads to the effect that the surface part of the kernel will be reduced in comparison to the interior part. As actually observed in baking tests with diploid and tetraploid Steel-rye, this leads to an increase of the bread volume with about 10 per cent and also for the baking of the hard Swedish »knäckebröd» the tetraploids are superior, judging from the preliminary reports obtained from large scale testing in a factory for making such bread. So far, baking tests have only been carried out with tetraploid Steel-rye and we don't know, how the other tetraploid strains will behave in this respect. However, at present this is of little importance as tetraploid Steel was found to be clearly superior to all the other strains so far involved in the trials.

After a careful consideration of the advantages and disadvantages of tetraploid Steel-rye it was recently decided to release this variety for practical use, especially on account of its baking quality, and, thus, in this autumn (1950) rather large propagations (about 300 acres) were sown in various places of South Sweden. Tetraploid Steel-rye will be available to the farmers in the autumn of 1951 or 1952. As far as I know, this is the first time induced polyploidy in a cereal has led to a product of economical importance. I am aware that in some respects this tetraploid is far from ideal, and in some years it will probably be replaced by new improved tetraploid varieties. Special expectations are attached to tetraploid King-rye on account of its rather short and very stiff straw. The first data from field trials of this variety will be obtained next summer. The properties of a tetraploid Petkus rye, produced in Germany, are also awaited with great interest.

In future work intercrossing of the best tetraploid varieties available will probably be of prime importance. Theoretically, such crossing-groups should be a more favourable material for selection than the »pure» strains, in which the number of genes is more limited. This argument may seem to be contradicted by the fact that among the material dealt with in the present paper the crossing-group is not *primus* but only *ultimus*, not reaching the standard of tetraploid Steel-rye. However, this crossing-group was made from four components of which two (American and Swedish × American) were later on found to be quite inferior and hence discarded. The crossing-group is clearly superior also to the third component, tetr. Wasa II, and only slightly inferior to the fourth component, tetr. Steel. At Ultuna, north of Stockholm, the crossing-group has even been found to be superior to tetraploid Steel. All this indicates that further work with intercrossing should be undertaken, but that it is desirable to start a new crossing-group with better components than the old one.

A weakness of the pure tetraploid varieties Steel and Wasa II is that they are constituted by a very limited number of tetraploid individuals (7 and 3 plants, respectively). Especially the results of HERIBERT NILSSON (1937) have taught us that every limitation of the gene supply, *i. e.* even the weakest ways of inbreeding, leads to a decrease in vigour and productivity. Hence, an improvement of tetraploid Steel-rye and tetraploid Wasa II will probably be obtained by producing new tetraploid individuals from the diploid varieties and including them in the present tetraploid strains. At any rate, it is certain that by various methods new and improved strains may be produced, and that these will be of greater importance than the material so far attained.

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SUMMARY.

The properties of seven varieties of tetraploid rye and some products of intercrossing were studied with the following main results:

(1) Meiosis in plants of four varieties showed a high frequency of quadrivalents at I-A, the average values ranging from 3,95 to 4,29. I-A is rather irregular with dividing univalents and other deviations from the normal distribution 14—14. The divided univalents lag at II-A and give rise to a rather high frequency of micronuclei in the tetrads. Studies of the tetrads indicate the degree of meiotic irregularity and the frequency of aneuploids in the material.

(2) The somatic chromosome numbers were studied in 808 plants. 77,23 per cent of the plants had $2n = 28$, 13,12 per cent $2n = 29$ and 7,43 per cent $2n = 27$. More extreme deviations were quite rare. Hyperploid aberrants are more frequent than hypoploids, and in one of the varieties the hypoploids were entirely absent.

(3) Cell size and plant height are greater in tetraploids than in diploids, the difference in height being about 15 per cent. No difference in strawstiffness could be established. The degree of tillering is reduced in the tetraploids, the average difference between diploid and tetraploid Steel-rye amounting to 12 per cent.

(4) The number of flowers per spike is somewhat lower in tetraploid than in diploid rye, and the tetraploids also have a somewhat greater tendency to shed the basal spikelets before maturity.

(5) Kernel weight in tetraploid rye is more than 50 per cent larger than in diploid rye. Hectolitre weight is 5—6 per cent lower, but this is a consequence of the large size of the kernals and not an indication of bad quality.

(6) In the best tetraploid varieties the average percentage of seed setting is about 62, the corresponding value of the diploids being approximately 80 per cent. Hence, seed setting in the tetraploids shows a reduction of about 20 to 25 per cent. Pollen fertility is apparently perfectly normal, most tetraploid plants having 90—100 per cent morphologically good pollen.

(7) There is a marked barrier of incompatibility between diploid and tetraploid rye, but a low proportion of triploid hybrids may be

obtained from both reciprocal combinations. Seed setting in tetraploid rye is much reduced, if the tetraploids are severely exposed to the pollen of ordinary rye. In such cases triploid zygotes are formed, most of which fail to develop into mature seeds. With increasing size of the tetraploid plots or fields the evil effects of haploid pollen is rapidly diminished.

(8) With regard to sprouting ability, measured by the percentage of seedlings per seeds sown, tetraploid rye is superior to ordinary rye. With regard to winter hardiness diploids and tetraploids are about equal, and in earliness there is no difference. Baking tests have been carried out with the result that tetraploid Steel has a higher protein content and gives a bread volume which is 10 per cent larger than that of diploid Steel.

(9) Kernel yield has been studied in eight years and in six different places in South and Central Sweden. Of the tetraploid varieties tetraploid Steel-rye is the most high-yielding one. Data obtained from 18 different trials failed to reveal any significant difference in yield between diploid and tetraploid Steel-rye. Thus, the big kernel size in the tetraploids and the superior sprouting ability compensate the reduced degree of seed setting, tillering, number of flowers per spike and the increased tendency to shed the basal spikelets.

(10) Especially on account of its good baking quality tetraploid Steel-rye is now propagated on a large scale and will be released to the farmers in the autumn of 1951 or 1952. Improved tetraploid varieties can be established, and future work on their production is outlined.

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THE MECHANISM OF CO-ORIENTATION IN BIVALENTS AND MULTIVALENTS

THE THEORY OF ORIENTATION BY PULLING

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I. INTRODUCTION.

THE present paper is devoted to an analysis of the *co-orientation* which takes place in bivalents and multivalents during meiosis. In order to analyse this phenomenon it is necessary also to consider a few other aspects of chromosome cytology in some detail. These other aspects, however, will be treated here only to the extent they contribute to an understanding of the co-orientation.

Thus, it is impossible to understand the behaviour of the paired chromosomes without also paying due regard to the *behaviour of univalent chromosomes* during the first division of meiosis. Some knowledge of the *structure of the kinetochore region* is also important for an understanding of certain features of chromosome behaviour, and there will be presented some important aspects of this subject in the present paper. Still, it was considered to be outside the scope of the present study to give a completely exhaustive discussion of the kinetochore structure here.

The co-orientation of the paired chromosomes is produced by the α-factor, as the poleward attraction of the kinetochores was called by ÖSTERGREN (1949 c). It was not considered necessary to review the evidence for the existence of the α-factor in the present connection, however. This evidence has been presented elsewhere (ÖSTERGREN, 1945 a, b, 1949 a, 1950 a; ÖSTERGREN and PRAKKEN, 1946; BÖÖK, 1945; PRAKKEN, 1948) and some of it will be presented in a more detailed form in another connection. The new results published in the present paper contribute also to give a considerable support to the idea of an α-factor working on the chromosomes.

II. ORIENTATION.

In a critical analysis of cytological events it is most useful to have a clear-cut terminology and then among other things to distinguish

sharply between, on one hand, terms intending to describe the *position* of bodies and, on the other hand, such intending to describe a relation of *direction* or *angle* of a body with reference to a certain system. It is in accordance with the current usage of the language to use the word »*orientation*» to describe the second kind of relation, and it would be very wise to restrict its use to this purpose only. (It might seem unnecessary to consider such obvious things here, but it actually happens now and then that cytologists speak of an »abnormal orientation» of a chromosome when they, instead, mean an abnormal position.)

If one accepts this significance of the term »orientation», then it will be useful to use the expression »the orientation of the kinetochores in a bivalent» in order to describe two different characteristics:

(1) It may be used to describe the *mutual* arrangement of the two kinetochores relative to the spindle. In normal cases the line passing through the kinetochores of a bivalent is parallel to the spindle axis (or perhaps more exactly, it coincides with the curved fibre arrangement of the spindle).

(2) If we assume or demonstrate that the *individual* kinetochore has a different constitution on different sides, then we may state that it is oriented in a special direction when it turns a special side in that direction.

There is a third property which one might consider to be denoted by the expression »the orientation of the kinetochore», *viz.* the direction of the force acting on the kinetochore. If a kinetochore is seen to be pulled towards one of the spindle poles, then it has sometimes been stated that the kinetochore is oriented towards this pole (without implying in this statement a constitutional difference of different sides of the kinetochore apparatus). It would be wise *not to use* the expression »the orientation of the kinetochore» in this sense, but to state simply that there is a force acting on the kinetochore in that direction. It seems most probable (*cf.* ÖSTERGREN, 1950 a, and see also below pp. 133—137 and 152) that the direction of the force is a consequence of the orientation of the constitutionally different sides of the kinetochore region. It might lead to confusions if this *consequence* of the kinetochore orientation was denominated by the same expression as the kinetochore orientation itself.

In a more restricted sense the word »orientation» may be used (as has often been done) to denote that kind of orientation which is *normal* for the chromosomes at mitosis and meiosis. In this sense one may state that a bivalent is »unoriented» when it has failed to

orient in the normal manner and that it is »oriented» when it has done so. There are two different kinds of orientation of the kinetochore apparatus of the chromosomes that normally take place during nuclear divisions. These have been called »co-orientation» and »auto-orientation» by DARLINGTON (1936 a, 1937 a, 1939 a). The meaning of these two terms will be explained below.

III. CO-ORIENTATION AND AUTO-ORIENTATION.

According to DARLINGTON (1936 a, 1937 a, 1939 a), there are three different processes which lead to the chromosome arrangement characteristic of full metaphase, *viz. orientation, congression* and *distribution.* These concepts seem quite useful and they will be accepted in the present connection (although the interpretation of the processes will be somewhat changed here).

There are two kinds of orientation, *viz. auto-orientation* and *co-orientation.* The most recent definition (DARLINGTON and MATHER, 1949, p. 407) runs as follows: »*Orientation*, the movement of the centromeres so that they lie axially with respect to the spindle, either as to their potential halves at mitosis (*Auto-orientation*) or as to members of a pair or higher configuration at meiosis (*Co-orientation*).» The expression »that they lie axially with respect to the spindle» shall, as explained by DARLINGTON (1936 a, p. 294), be taken to mean that the line passing through the potential kinetochore halves (at mitosis) or the two partner kinetochores in a bivalent (at meiosis) coincides with the curved fibre arrangement of the spindle; they lie on the same spindle arc.

The expression »congression» is defined as follows (DARLINGTON, 1937 a, p. 575): »*Congression*, the movement of the chromosomes on to the metaphase plate.» The *distribution* is the process by which the chromosomes spread over the metaphase plate in an arrangement showing certain regularities. DARLINGTON (1937 a, p. 524) considers the body repulsion to be one of the main causes of this distribution. The term can naturally be used without the acceptance of any assumptions concerning the causes of the process in question.

According to the interpretations of ÖSTERGREN (1950 a), the kinetochore region of the chromosomes at the first division of meiosis has a constitutional differentiation of its different sides, it has what may be called a kinetic (or front) side and an akinetic (or back) side. The correctness of this opinion is demonstrated by direct observations on

chromosome structure (*cf.* below). The idea of such a dorsiventral structure of the kinetochore region has earlier been expressed by LORBEER (1934), who also used the expressions »Vorderseite» and »Rückseite», and this opinion was also taken up in the review of GEITLER (1938 a, p. 17).

The behaviour of the chromosomes suggests (*cf.* ÖSTERGREN, 1950 a) that the two kinetochores of each bivalent are oriented with their front sides towards opposite poles during metaphase. Each kinetochore is attracted only towards that pole towards which its front side is oriented, and the forces of attraction are of such kind that they grow stronger with increasing distance between the kinetochore and the spindle pole. This attraction was called the α-factor of chromosome behaviour by ÖSTERGREN (1949 c). How this attraction is produced by the spindle is unknown (*cf.*, however, the speculative interpretations by ÖSTERGREN, 1949 b). In the present connection, however, we need not worry about the mechanical nature of these forces, but we can accept their existence and proceed to analyse the mechanism of the co-orientation in terms of these forces.

The attraction of the two kinetochores of a bivalent towards the opposite spindle poles orients the bivalent in such a way that the line passing through the kinetochores becomes parallel to the spindle axis, and this same attraction brings the chromosomes to the metaphase plate in the congression movement. After the separation of the partner chromosomes the same attraction produces the anaphase movement of the chromosomes to the poles. So far, the hypothesis gives an easy explanation of the co-orientation. Naturally, the real problem arises when we start to consider *how the two partner kinetochores of the bivalents come to orient their kinetic sides towards opposite poles.* This orientation of the partner kinetochores towards opposite poles actually constitutes the *essential feature* of the co-orientation; the orientation of the bivalent as a whole is a simple consequence of this effect. DARLINGTON who tried to explain the behaviour of the chromosomes on the basis of quite a different hypothesis had no idea of the existence of this effect (at any rate he has not mentioned it). Consequently it may be suitable to re-define the co-orientation concept when attention is paid to this effect.

I suggest the following definition: *Co-orientation* is the process of *mutually correlated orientation* of the kinetochores of chromosomes that are involved in a bivalent or multivalent. Through this process the kinetochores of the bivalents come to be oriented with their kinetic

sides directed towards opposite spindle poles. And in the multivalents there will be a preferential orientation of neighbouring kinetochores towards opposite poles. The kinetochores oriented towards opposite poles are pulled in these opposite directions and they will as a consequence orient the bivalents and multivalents in their characteristic arrangements.

Obviously the co-orientation must be due to some kind of *mutual interaction between the kinetochores* involved. The direction of the forces acting on the kinetochores demonstrates in itself the existence of some kind of interaction between the kinetochores. In the absence of an interaction the forces acting on the two chromosomes of a bivalent should naturally just as often have been directed towards the same pole as towards opposite ones. The existence of such an interaction of one kind or another between the kinetochores must be regarded as a conclusion that is definitely proved. This conclusion is independent of what other interpretations we may suggest concerning the nature and properties of the forces moving the chromosomes.

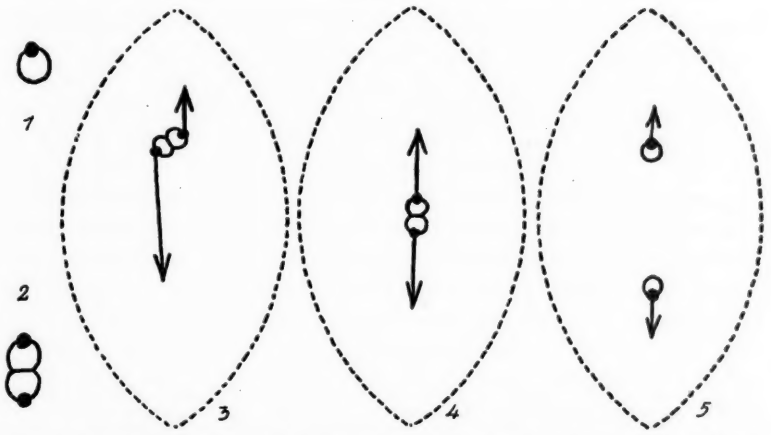
On this point of an interaction between the kinetochores in bivalents and multivalents one might be tempted to raise doubts on the basis that it is very difficult to conceive how the two partner kinetochores in long rod bivalents could possibly influence one another over the long distance separating them (a distance sometimes amounting to or even exceeding 20 μ, *e. g.* in *Lilium*, Figs. 139—141). But such doubts need not be taken up for discussion. The circumstances are such that the question cannot be *if* there exists such an interaction, but one must instead ask *how* this interaction works and how it is capable of doing so even in very long rod bivalents where the kinetochores are very widely separated. The fact that the interaction can work even in such cases is a point that must be considered in any hypothesis formulated to explain the behaviour of bivalents and multivalents.

Before we start to analyse the details of the mechanism of the co-orientation it may be useful to insert a few words on the *auto-orientation*. As analysed by ÖSTERGREN (1950 a), the *metaphase equilibrium* of the kinetochores half-way between the two spindle poles suggests that the kinetochores are attracted to the poles by forces increasing in strength with increasing distance between the kinetochore and the pole (*cf.* also ÖSTERGREN, 1945 a and b; ÖSTERGREN and PRAKKEN, 1946). The most probable interpretation is that the anaphase movement is due essentially to the continued action of these same forces (the *a*-factor) which acted on the kinetochores already during metaphase. But if such forces are to be able to move the kinetochores out of the

equatorial position at all, it will be necessary to assume that the interaction between the *anaphase* kinetochore and the spindle is of an asymmetric kind, *i. e.* the anaphase kinetochore has what may be called a front (or kinetic) side and a back (or akinetic) side. There is evidence for these structural properties of the kinetochore from direct morphological observations also (*e. g.* SCHRADER, 1936). Under these circumstances the behaviour of the *metaphase kinetochore of mitosis* will be most easily explained by the assumption that it *consists essentially of two anaphase kinetochores* attached to one another by their akinetic sides and turning their kinetic sides in opposite directions (Figs. 1—2). The correctness of these interpretations is demonstrated by direct observations on kinetochore structure and by the attachment of the chromosomal spindle fibres on the kinetochores (SCHRADER, 1936; ÖSTERGREN, 1947 a). The study by SCHRADER (1936) was made on meiosis of *Amphiuma*. The stage which especially interests us in the present connection is the second division, as the kinetochore behaviour is of ordinary mitotic type during this division. SCHRADER observed that each metaphase chromosome had two spindle spherules, corresponding to the two chromatids. The two spindle spherules were lying on the two opposite poleward sides of the chromosome and each of them was furnished with fibre structure towards that pole towards which it was turned. Thus, the kinetic component of the kinetochore (the »spindle spherule») is divided already at metaphase, and the association of the two chromatids in this region must be due to some other factor (*cf.* SCHRADER, 1939, p. 236). SCHRADER also makes a brief note that the chromosomes are organized in this way also in ordinary mitotic divisions (1936, p. 492). Similar observations on kinetochore structure were made on root tip mitoses of various plants by ÖSTERGREN (1947 a) and by TJIO and LEVAN (1950 a and b); in both cases special treatments were applied to the root tips.

On the basis of such a structure of the mitotic metaphase kinetochore the process of *auto-orientation* will be easily understood (Figs. 3—5). The metaphase kinetochore has two kinetic sides represented by the two »spindle spherules» localized on opposite sides of it. These sides are attracted towards those poles towards which they are chiefly oriented in their originally random arrangement; and these attraction forces will automatically orient the two kinetic sides towards opposite poles. Only the *auto-oriented* arrangement will represent a stable equilibrium.

It has occasionally been seen in divisions of mitotic type even



Figs. 1—5. Diagrams illustrating the mechanism of *auto-orientation* in ordinary mitotic divisions. — Fig. 1. Cross section of a kinetochore at anaphase of mitosis. On one side of it the kinetic component (the »spindle spherule»); this is the kinetic side or front side of the kinetochore. — Fig. 2. The metaphase kinetochore consists essentially of two anaphase kinetochores attached to one another at their akinetic sides. — Fig. 3. During prometaphase the kinetochore has originally been localized and oriented at random. Its two kinetic sides are attracted towards those poles towards which they are chiefly oriented. This attraction produces the auto-orientation of the kinetochore and brings it to the equator. — Fig. 4. The metaphase equilibrium. — Fig. 5. When the two daughter kinetochores have separated, the continued action of the same poleward attraction produces the anaphase movement.

in cases where no special treatment was applied, that the kinetic component (the »spindle spherule», the »centrogene», or what we may like to call it) is divided already at metaphase. This was seen by BARBER (1941) in the pollen grain mitosis of *Uvularia* and by HUGHES-SCHRADER (1947) in the second meiotic division of *Isagoras subaquilis*. The *Amphiuma* case of SCHRADER mentioned above may also be referred to here. Obviously these cases are not any exceptions to the ordinary rules of kinetochore structure, but they are simply cases where the general principle of the doubleness of the metaphase kinetochore is more easily visible than it usually is.

The double structure of each one of the two »spindle spherules» of the metaphase chromosome which was observed by ÖSTERGREN (1947 a) and by TJIO and LEVAN (1950 a and b) is no obstacle to the present interpretations. The two halves of each spindle spherule appear to be closely associated (in studies by various other investigators this doubleness was overlooked) and these halves are also oriented in the

same direction. For these reasons the two halves may be considered to form a functional unit in the chromosomes of ordinary mitosis.

DARLINGTON's idea that the mitotic »centromere» is undivided and that the orientation at metaphase is one of »potential halves» has no evidence in support of it. DARLINGTON assumes a *functional doubleness* of an *undivided* »centromere» at mitotic metaphase (it is supposed that the body in question becomes »hour-glass shaped»; DARLINGTON, 1937 a, p. 539). These speculations seem scarcely justified. It is by far the simplest interpretation to assume that the kinetic component (the spindle spherule of the kinetochore) has at metaphase a *doubleness* which is not only functional but also *structural.*

IV. STRUCTURE OF THE KINETOCHORE REGION DURING THE FIRST DIVISION OF MEIOSIS.

1. DISCUSSION.

Above it was stated that the kinetochore region of each chromosome at the first division of meiosis has a constitutional difference of its different sides; it has a *kinetic* and an *akinetic* side. Furthermore, it was stated that the two kinetochores of each co-oriented bivalent at first metaphase are oriented with their kinetic sides towards opposite poles. The fundamental feature of the co-orientation was actually considered to be an interaction between the two kinetochores of each bivalent of such kind as to orient them in this way towards opposite poles. This interpretation of kinetochore structure and behaviour can be extracted from the behaviour of the chromosomes (ÖSTERGREN, 1950 a). But naturally the question arises whether this picture is also supported by direct *observations on chromosome structure.*

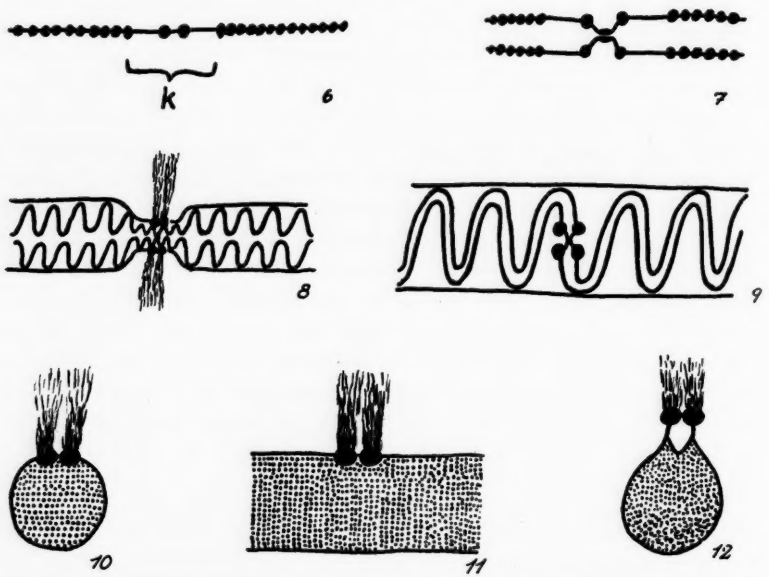
The metaphase chromosomes are constituted by threadlike chromonemata that have assumed the form of a spiral coil and that have been enveloped in a nucleoprotein cover, which (according to some authors) may be called a matrix. For this reason it is suitable first to consider the chromosome in its extended (chromonema) state when we try to analyse the structure of the kinetochore. LIMA-DE-FARIA (1949, 1950) has analysed the kinetochore structure in pachytene chromosomes of *Secale cereale* and *Agapanthus umbellatus.* He found that each kinetochore contains the following three regions (Fig. 6) counted from the chromosome arms and towards the centre of the kinetochore: (1) An exterior zone of fibrillar structure. (2) A pair of chromomeres, the

»centromeric chromomeres». (3) Between these two chromomeres, in the very centre of the kinetochore, there is a third zone, an interchromomeric connection between the two centromeric chromomeres.

The same organization of the kinetochore region could be very clearly observed by TJIO and LEVAN (1950 a and b) in somatic metaphase chromosomes from root tips of numerous plant species. TJIO and LEVAN applied a special technique involving treatment with 8-oxyquinoline. It was found that the kinetochore region is divided into two chromatids just like the rest of the chromosome. It contains two fibrils corresponding to the two chromatids and each of these fibrils contains a pair of »centromeric chromomeres», *i. e.* each chromatid is organized quite as was found by LIMA-DE-FARIA for the pachytene chromosome. Similar observations were made by ÖSTERGREN (1947 a), who also observed that these centromeric chromomeres[1] were attached to the spindle fibres, for which reason they may be considered to be the actively mobile components of the kinetochore. No doubt, these »centromeric chromomeres» correspond to the »spindle spherules» of SCHRADER (1936, 1939), which thus (in many cases, at least) must have an internal doubleness in the longitudinal direction of the chromosome.

In many organisms there is an important difference in the structure of the kinetochore region between ordinary mitosis and the first division of meiosis, a difference which (as far as I know) has never been pointed out by any investigator, although it must have been observed many times, as is clear from drawings published by various authors. The difference consists in the fact that the kinetochore region in ordinary *mitotic* chromosomes is *constricted* while, on the other hand, the *spiralization* of this region *does not differ* (or differs but little) from that of the rest of the chromosome during the *first division of meiosis*. Or, in other words, the kinetochore shows *negative heteropycnosis* in ordinary mitosis, but in the first division of meiosis it is *isopycnotic* (a term coined by ÖSTERGREN, 1950 b). In some other species, however, the kinetochore region shows negative heteropycnosis during the first meiotic division also (see, further, the study on various species reported later in this paper). Although the difference is accordingly not of general validity, it is still an important one in showing us a difference in the principle of organization of mitotic and meiotic chromosomes. It is probable that a difference between mitotic and

[1] The assumption by ÖSTERGREN (1947 a) that these centromeric chromomeres might be identical with the »proximal heterochromatin» of LEVAN (1946) is, I think, a mistake.



Figs. 6—12. Diagrams showing the structure of the kinetochore region. — Fig. 6. Structure of the chromonema as found in the pachytene studies by LIMA-DE-FARIA. The kinetochore is denoted by *k*. — Fig. 7. The basic structure of a metaphase chromosome. The spirals have been disregarded in this scheme. Most probably the two chromonemata are especially intimately associated in the central region of the kinetochore. — Figs. 8—9. Two types of spiralized metaphase chromosomes, both having the basic structure of Fig. 7. — Fig. 8. A metaphase chromosome of ordinary *mitosis*. Such a chromosome shows a kinetic constriction and has the kinetic granules arranged on two opposite sides of the chromosome. The chromosomal spindle fibres are drawn. — Fig. 9. A metaphase chromosome of the first division of *meiosis*. There is no kinetic constriction and all the kinetic granules consequently become arranged on one side of the chromosome body. — Fig. 10. A cross section through a chromosome of this kind. — Fig. 11. The same chromosome in longitudinal section. — Fig. 12. A cross section similar to Fig. 10, but in this case the kinetochore has been drawn out above the chromosome surface by the poleward pull. — In Figs. 10—12 the chromosomal spindle fibres have been drawn.

meiotic chromosomes of the same kind, although smaller, also exists in those species that show a kinetochore constriction during the first division. Below, data will be presented showing that the strongest exception to this rule actually is of such nature as to reinforce the general conclusions of the present paper (*Tulipa*, p. 106).

In the narrow kinetochore region of an ordinary metaphase chromosome of mitosis there are good possibilities for the centromeric chromomeres of the two chromatids to arrange themselves on opposite

sides of the kinetochore. This arrangement may be brought about by spiralization as suggested in Figs. 7—8. In these figures I have assumed, as suggested by LIMA-DE-FARIA (1949), that the component which keeps the two sister kinetochores closely associated is located in the most central part of the kinetochore region. The characteristic pictures obtained by TJIO and LEVAN from oxyquinoline treatment are probably derived from the kinetochore type of Fig. 8 by simple stretching. As suggested by these authors (TJIO and LEVAN, 1950 b), the chromosome arms may be fixed in the solidified cytoplasm and when the chromosomes contract the kinetochore region is stretched.

As is well-known, the sister kinetochores are associated with one another until the onset of anaphase during ordinary mitosis and until that of second anaphase during meiosis. It is most probable that this association takes place in the most central part of the kinetochore (called region 3 above, pp. 92—93). But it is not necessary in the present connection to consider in detail the mechanism by means of which this association may be produced. It may result from the presence inside the kinetochore of some special component which is undivided until the start of anaphase, or more probably it is just a variant of the general phenomenon of »chromatid attraction» (or »chromatid association»). There is usually a characteristic lapse of chromatid association in the chromosome arms at the onset of anaphase both in ordinary mitosis and in the first division of meiosis. A similar change may take place in the kinetochore at the time when it »divides», as it has been called. There is no reason to assume that the components of the kinetochore make their real division or reproduction at another time than the other components of the chromonema; and in the absence of evidence on this point an identical time of division seems more probable than a different one.

At the first metaphase of meiosis the kinetochore region is (in many materials, at least) spiralized in the same way as the rest of the chromosome. There is in such a case no possibility for the kinetic chromomeres, which are so close together in the two chromonemata, to occupy positions on opposite sides of the chromosome. Thus, the isopycnosis of the kinetochore region at first metaphase is in itself quite sufficient to produce a one-sided localization of the kinetic components of the chromosome. In the present connection it should also be considered that the whole chromosome is much broader during the first division than it is at ordinary mitosis; the spiral gyres have a larger radius. This fact makes the possibility for a two-sided localization of the kinetic components even smaller. One can with a high degree of safety conclude that the whole kinetochore must be localized on one side of the chromosome (Figs. 9—11).

(I have in various connections expressed the opinion that at the first division of meiosis the kinetochore has a *kinetic* and an *akinetic*

side. In the case of such chromosomes as have an isopycnotic kinetochore region this statement may be appropriate *only from a functional standpoint*; when more attention is paid to the above-mentioned features of the chromosome organization, then it might be better to say that the whole kinetochore is localized on one side of the chromosome. It is the *chromosome* rather than the kinetochore which has a one-sided localization of the kinetic properties. Or one might say that the chromosome body is screening off the kinetochore so that it can interact well with the spindle in one direction only.)

In the above reasoning the conclusion of a one-sided localization of the kinetic properties in the chromosomes of the first division of meiosis was derived from (1) the constitution of the pachytene chromosomes studied by LIMA-DE-FARIA and the related observations on the mitotic kinetochore structure made by ÖSTERGREN (1947 a) and by TJIO and LEVAN (1950 a and b), and from (2) the isopycnosis of the meiotic kinetochore region. There is, however, also evidence of a more direct kind supporting this opinion, *viz.* from observations on the structure of the kinetochore region and the attachment of the spindle fibres. Such observations were made by SCHRADER (1936, 1939) and by DARLINGTON (1937 a and b); some more of a similar kind will be presented below by the present writer (pp. 109 and 132—133).

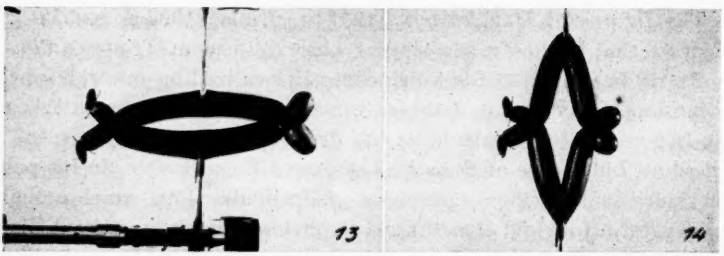
SCHRADER (1936, 1939) observed in the bivalents of the first meiotic division of *Amphiuma* that the kinetochore is lying on one side of the chromosome body. He did not see four kinetic bodies as suggested in my scheme (Fig. 9), but only two »spindle spherules» corresponding to the two chromatids. The two halves of these spindle spherules may have been too closely associated to be distinguishable, or there is also a possibility that this material has a real organizational difference in this respect from such materials as rye (*cf.* LIMA-DE-FARIA, 1949). But the fundamental principle of the one-sided arrangement of the kinetic component is clearly demonstrated in these studies of SCHRADER. It should also be mentioned that the kinetochores in these bivalents were found to be oriented towards opposite poles at metaphase and furnished with spindle fibres directed towards the poles towards which they were oriented. The chromosomal spindle fibres were attached to the spindle spherules. In *Amphiuma* the spindle spherules were located in a »commissural cup» (SCHRADER, 1936, 1939), an arrangement which probably means that the kinetochore region shows some degree of negative heteropycnosis in this material during the first division of meiosis also.

The figures of DARLINGTON (1937 b; *cf.* also 1937 a, p. 528) demonstrate that he has made similar observations on *Tradescantia.* It can clearly be seen that the kinetochore is localized on one side of the chromosome body and that the chromosome is isopycnotic in this region. In some of the bivalents he has drawn the »spindle spherules» as single dots, but in one of them he has drawn them double. In his polar view figure some of them appear as quadrangular dots, which actually may suggest a fourfold structure. It is obvious that it must be difficult to see clearly if such small structures are single, double or multiple, as pointed out by DARLINGTON himself (1939 b). SCHRADER (1939) studied *Tradescantia,* too, and he frequently observed a division in two of the kinetic granules (*i. e.* two kinetic bodies in one chromosome at first metaphase). He even found indications (though not convincing him) of further subdivisions, as previously seen by NEBEL (1935). The figures of LACOUR (1935; *cf.* also DARLINGTON, 1937 b; and 1937 a, p. 488) showing the spiral structure of *Tradescantia* chromosomes at first metaphase demonstrate that the kinetochore region is spiralized in the same way as the rest of the chromosome. The same feature can also be seen in the figures of RUCH (1949; also confirmed by personal information to me from Dr. RUCH). Furthermore, a few observations on *Tradescantia* made by the present writer are given below.

Undoubtedly there could be found many other figures in the literature which also demonstrate the isopycnosis of the kinetochore region during the first division of meiosis, but the references given may suffice for the moment.

There are other materials which show a clear kinetochore constriction at the first division; here belong *Allium* and *Tulipa; cf.* further below. No doubt, however, there have been published drawings by some authors which show kinetochore constrictions much more clearly than they exist in their slides. There is a general belief that it is a fundamental feature of all chromosomes to have a kinetochore constriction, and some authors have idealized their figures so that these show what the authors think a chromosome should look like rather than how the chromosome actually appeared.

At first metaphase there is a feature of the chromosomes which easily gives rise to misinterpretations in this respect, *viz.* the mechanical deformation to which the chromosomes are subjected from the poleward pull working on the kinetochores. Because of this poleward pull ring bivalents are often folded in a conspicuous manner in their kinetochore regions, and these folds might easily be mistaken for constrictions



Figs. 13—14. — Chromosome models of rubber balloons showing the mechanical deformation of the chromosomes at first metaphase. — Fig. 13. Diakinesis. The chromosomes are free from kinetochore forces and show no kinetic constrictions. — Fig. 14. First metaphase. The poleward pull working on the kinetochores results in characteristic folds of the chromosomes that might easily be mistaken for kinetic constrictions. The model is strikingly similar to a metaphase bivalent.

(Figs. 13—14). The nature of this fold is easily found out by a study of the chromosomes at diakinesis, when they are free from the influence of these spindle forces. It is a characteristic feature of the chromosomes at diakinesis in the most various materials that they do not show any kinetochore constrictions (see, *e. g.*, the figures in the book of DARLINGTON, 1937 a). The real structure of the kinetochore region can also be found out by a study of univalents as well as, sometimes, of rod bivalents (*cf.* below). In these chromosomes the mechanical deformation caused by the mitotic forces is not so great that it will cause disturbances.

There is a characteristic feature of the chromosomes during the first division of meiosis which may be mentioned in the present connection. It is often found that the centromeric chromomeres or spindle spherules (or what we may choose to call them) are drawn out on long fibrils above the chromosome surface during the first metaphase of meiosis, but that they are not drawn out in this manner during the ordinary mitotic divisions (*cf.*, *e. g.*, SCHRADER, 1939). This difference is easily understood if we consider that the load of the poleward pull is carried by quite different components in ordinary mitotic chromosomes and in paired chromosomes during the first division of meiosis. During metaphase of a mitotic division the load of the poleward pull working on the centromeric chromomeres will be carried by the interchromomeric region (zone 3, p. 93) in the central part of the kinetochore (*cf.* Figs. 6—12). But, at the first metaphase of meiosis this load will be carried by the fibrils (zone 1) which connect the centromeric chromomeres with the chromosome arms. The studies of LIMA-DE-FARIA demonstrate that in his materials zone 1 is much longer than zone 3 (and it is reasonable to suppose that this is also the case in many other materials). Consequently it is not surprising that zone 1 will be much longer than zone 3 also when it is attenuated by the mitotic forces, and it will be easy to understand why the spindle spherules may be seen on

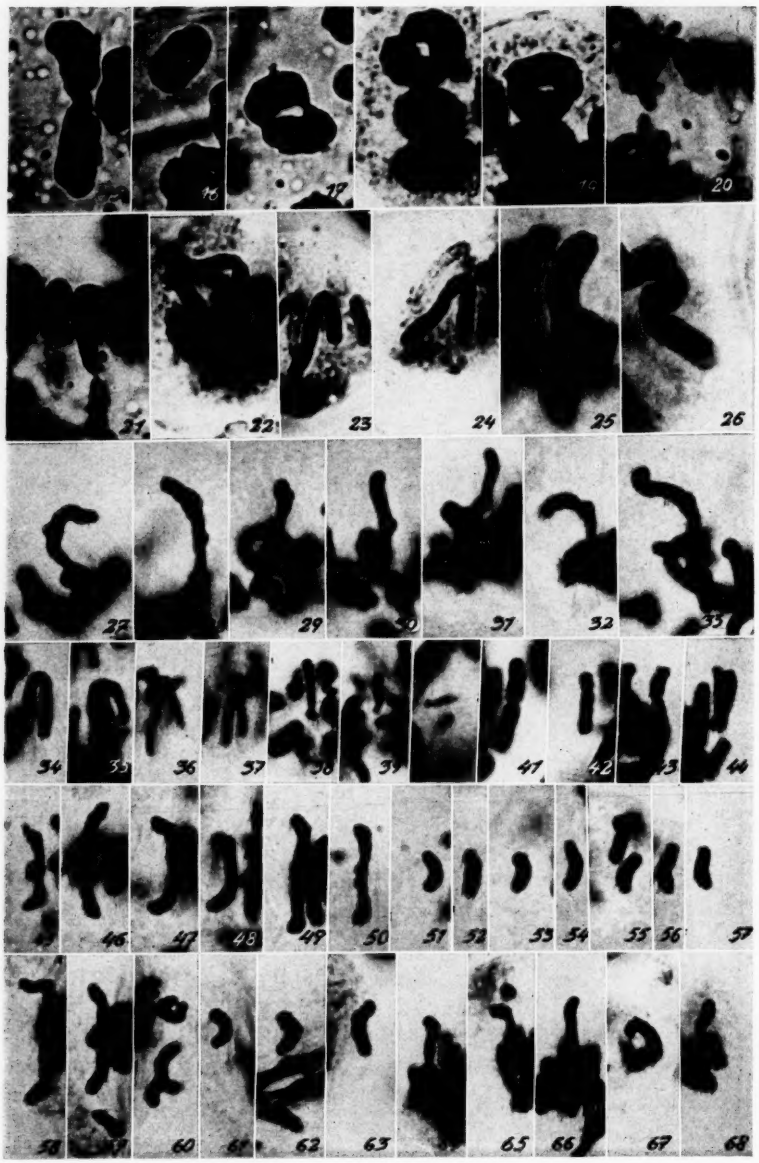
attenuated fibrils during first metaphase but not during ordinary mitosis. The alternative suggestion made by SCHRADER (1939) does not seem probable at all. He suggested that the protrusion of the spindle spherules at the first meiotic division was due to a higher resistance to anaphase separation of the chromosomes because of the chiasmata and the end attraction. To this it must be objected, however, that the initial separation of the paired chromosomes in the first division is not due to an overcoming of the chromosome association by the poleward pull; instead it follows from a sudden lapse of the chromatid association at the onset of anaphase, a lapse due to internal chromosome changes of some kind. This characteristic »lapse of chromatid attraction» takes place simultaneously in bivalents and in unpaired chromosomes (*cf.* GEITLER, 1938 b; and ÖSTERGREN, unpublished).

2. VARIOUS OBSERVATIONS.

In order to test the above considerations I have undertaken a comparative study of the appearance of the kinetochore region during the first meiotic division in a number of plants. Some materials do not show the slightest tendency to a kinetic constriction during first metaphase, while others actually show more or less distinct primary constrictions. The results are presented in a condensed form in Table 1. The exact meaning of the different degrees of classification used in this table is clear from the photographs (Figs. 15—127).

The material used in this study consisted in many cases of hybrids or triploids. Such material was used in order to get univalents. Even univalents are subjected to deformation by the mitotic forces (*cf.* below), but these deformations are so weak that they do not conceal the structural properties of the kinetochore region. In ring bivalents the mechanical deformation is usually very disturbing (*cf.* Figs. 13—14), but rod bivalents are often quite useful for the present purpose, and this is also the case with chromosomes taking part in trivalents, if these chromosomes have a chiasma on only one side of the kinetochore (such chromosomes were studied especially in *Hyacinthus*, Figs. 27—33, and in *Butomus*, Figs. 64—68). In *Tradescantia* even ring bivalents are quite good for the present study.

In the case of much of this material the characteristic features of the kinetochore region in the meiotic chromosomes can also be seen from various figures published by other authors (*e. g.* for *Tradescantia* see the references given above, and for *Secale cf.* MÜNTZING and PRAKKEN, 1941; PRAKKEN and MÜNTZING, 1942; PRAKKEN, 1943; MÜNTZING and AKDIK, 1948). As far as I know, there has, however, never been made any special study of the aspect considered here. As regards the general cytological features of the materials studied (frequencies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of bivalents, trivalents, *etc.*), they are of no interest in the present connection and will be disregarded here.

It might be suggested that the absence or weak development of the kinetic constriction in the chromosomes at first metaphase is only apparent, *i. e.* there is not any real organizational difference between mitotic and meiotic chromosomes in this respect, but the constriction in the meiotic chromosomes is merely concealed in some way by the very strong contraction of these chromosomes. This objection can be easily disproved, however, by a study of supercontracted chromosomes from mitotic divisions. It is a well-known fact that somatic chromosomes which have been supercontracted by treatment with cold or with various chemicals actually show their kinetic constrictions much more clearly than ordinary mitotic chromosomes. This demonstrates that there must be a real organizational difference of the kind suggested

Figs. 15—68. Photographs of chromosome structure in *Tradescantia, Trillium, Hyacinthus, Secale, Triticum,* and *Butomus.* — Figs. 15—24. *Tradescantia.* — Figs. 15—17 and 20—21. *T. virginiana,* and Figs. 18—19 and 22—24. *T. paludosa.* — Figs. 15—19. First metaphase and prometaphase showing the absence of kinetic constrictions. In the bivalents of Figs. 15 and 17 the kinetochores are protruding (they are visible in the upper chromosomes). — Figs. 20—24. Second anaphase showing V-bent chromosomes having kinetic constrictions. — Figs. 25—26. *Trillium erectum.* Chromosomes at first (Fig. 25) and second (Fig. 26) metaphase. The kinetochore region is not constricted at first metaphase (see the upper chromosome), but it is slightly constricted in second metaphase. — Figs. 27—44. *Hyacinthus orientalis* (triploid). — Figs. 27—33. Chromosomes taking part in trivalents at first metaphase do not show any kinetic constrictions. — Figs. 34—35. First anaphase chromosomes are bent in U-shape. — Figs. 36—37. Second anaphase chromosomes are bent in V-shape. — Figs. 38—39. Second metaphase chromosomes in polar view show kinetic constrictions. — Fig. 40. Lagging daughter univalent in second anaphase shows kinetic constriction. — Figs. 41—44. Supercontracted somatic chromosomes show distinct kinetic constrictions. — Figs. 41—42. Chromosomes in floral tissue treated with 1 % colchicine (24 hours). — Figs. 43—44. Root tip chromosomes treated with 0,05 % colchicine (24 hours). — Figs. 45—57. First metaphase in *Secale cereale* (a partially asynaptic plant from »Östgöta Gråråg»). — Figs. 45—50. Rod bivalents. — Figs. 51—57. Univalents. — Figs. 45—57 do not show any primary constrictions, but a secondary constriction is clearly visible in Fig. 45 and may be indicated in Fig. 52; the bivalent of Fig. 46 probably shows tendencies of T-effect (*cf.* p. 108). — Figs. 58—61. Rod bivalents and univalents of *Triticum vulgare* × *turgidum* (F_2) do not show any kinetic constrictions at first metaphase. — Figs. 62—63. Univalents from *Triticum vulgare* × *Secale cereale* showing absence of kinetic constrictions at first metaphase. — Figs. 64—68. *Butomus umbellatus* (triploid or ± triploid). Chromosomes taking part in trivalents or bivalents do not show any kinetic constrictions. — Figs. 15—26. Fixed and stained in acetocarmine. Smears. — Figs. 27—68. Fixed in chrome-acetic-formalin after prefixation in acetic alcohol (with the exceptions of the root tips, Figs. 43—44, that were not prefixed). — Figs. 27—42 and 45—68. Microtome sections. — Figs. 43—44. — Squash preparation. — Figs. 27—40 and 45—57. Crystal violet. — Figs. 41—44. Feulgen. — Figs. 58—68. Acetocarmine and crystal violet, double stain. — All figures × 1500.

TABLE 1. *The kinetochore constriction during first metaphase in various species.*

A. No kinetic constriction (isopycnotic kinetochore region).
Tradescantia virginiana
Tradescantia paludosa
Trillium erectum
Hyacinthus orientalis (triploid)
Secale cereale (normal, partially asynaptic, triploid, haploid)
Triticum vulgare × *turgidum* (an F_2 plant)
Triticum vulgare × *Secale cereale* (F_1 and amphidiploid)
Butomus umbellatus (triploid or ± triploid)

B. Indications of kinetic constrictions.
Lilium candidum × *chalcedonicum* (»*L. testaceum*»)
Lilium tigrinum (triploid)
Anthoxanthum aristatum (triploid)

C. Distinct, but not very pronounced kinetic constrictions.
Paeonia anomala × *tenuifolia*
Paeonia officinale
Allium Cepa × *fistulosum*

D. Pronounced kinetic constrictions.
Tulipa Gesneriana (triploid)

Note: Also in the rye and wheat materials there may in a low frequency of chromosomes be some weak indications of kinetic constrictions. — Concerning details on materials and methods, see pp. 148—149.

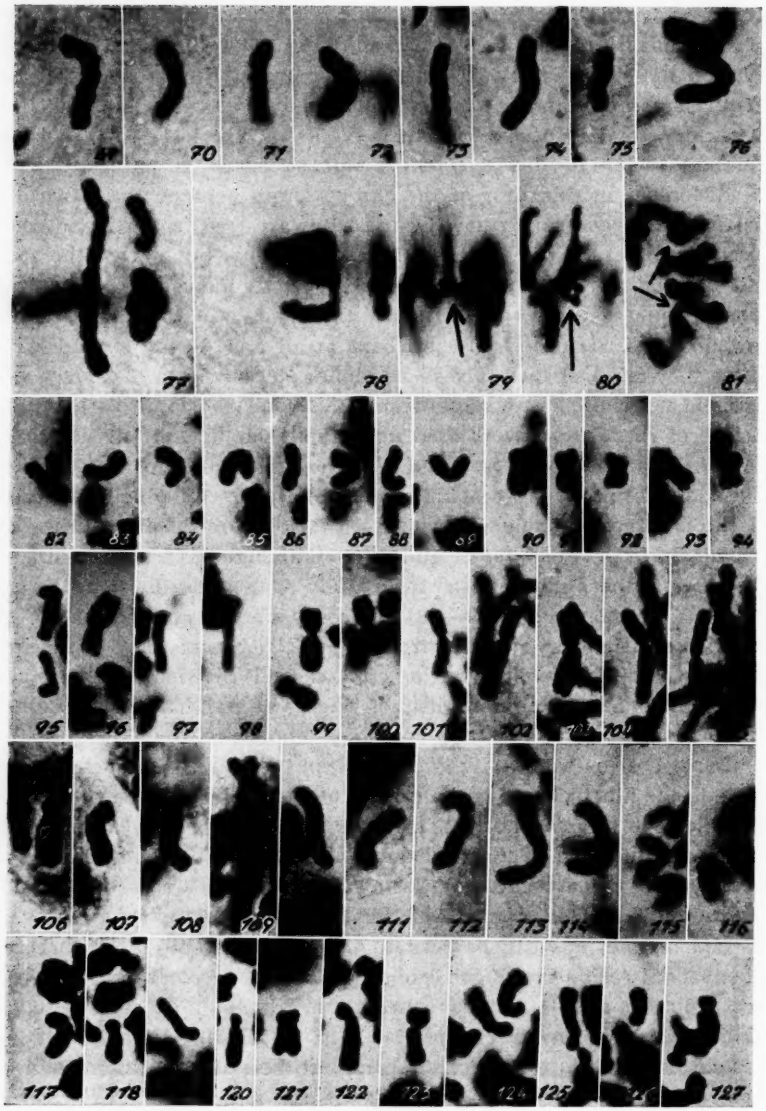
here between the chromosomes of ordinary mitosis and those of the first meiotic division.

Such supercontracted somatic chromosomes have been illustrated in Figs. 41—44 and 96—105 for comparison with the meiotic chromosomes. In *Hyacinthus* the chromosomes of the first metaphase do not show any kinetic constrictions at all (Figs. 27—33), but the somatic chromosomes of Figs. 41—44 show them very clearly. The cells of Figs. 41—42 are from somatic tissue of flower buds. They have been fixed in identically the same way as the first metaphase chromosomes of Figs. 27—33. So an interference from differences in fixation is excluded.

Some comparisons between the chromosomes of the first and the second divisions of meiosis were also made. In *Hyacinthus*, where there

is no constriction at first metaphase, the chromosomes show kinetic constrictions during the second division (Figs. 38—40). The difference between these two divisions is also demonstrated by the shape of the anaphase chromosomes; at first anaphase they are bent in U-shape, but at second anaphase they are bent in V-shape (Figs. 34—37). (A contributing factor to this different anaphase shape is naturally also the difference in thickness of the chromosomes between these two divisions.) In *Tradescantia* the chromosomes are free from kinetic constrictions and very thick during the first division, but during the second division they are narrow and show clear indications of centric constrictions (Figs. 15—24). During first anaphase the *Tradescantia* chromosomes are practically not bent at all and during second anaphase they are V-shaped. My slides of *Lilium candidum* × *chalcedonicum* did not contain any stages from the second division, so second divisions of *L. tigrinum* were used for comparison. The strong deformation of the paired chromosomes during the first division of *L. tigrinum*, caused by the mitotic forces, made this material less suitable for a study of the chromosome shape during the first division. The frequency of univalents in *L. tigrinum* is quite low (although it is a triploid), so the other lily was mainly used for a study of the first division. During the first division (Figs. 69—78) there are only weak indications of kinetic constrictions (slightly stronger in *L. tigrinum* than in *L. candidum* × *chalcedonicum*). During the second division the primary constrictions are much more distinct (Figs. 79—81). The chromosomes are V-bent in second anaphase and show tendencies to a V-bend in the first anaphase as well. In *Trillium erectum* there are usually chiasmata close to the kinetochores on both sides of them. These chiasmata make it difficult to observe the real structure of the kinetochore region. This structure can be well observed only in those few bivalents that have happened to get a reduced frequency of chiasmata or an unusual localization of them. A bivalent of this kind is photographed in Fig. 25. It demonstrates (as can be seen in the upper chromosome) that the kinetochore region is not constricted. During the second division there are indications of a kinetic constriction, although by no means so distinct as might have been expected (Fig. 26). During second anaphase the chromosomes are distinctly V-bent, but there are indications of a V-bend also in first anaphase. Only a small material of *Trillium* was studied, and I think it would be desirable to extend the studies before definite conclusions are made.

In the great majority of cases the chromosomes of *Secale cereale*



Figs. 69—127. Photographs of chromosome structure in *Lilium*, *Allium*, *Paeonia* and *Tulipa*. — Figs. 69—81. *Lilium*. — Figs. 69—78. *L. candidum* × *chalcedonicum*. First metaphase univalents (Figs. 69—76) and bivalents (Figs. 77—78) showing only weak

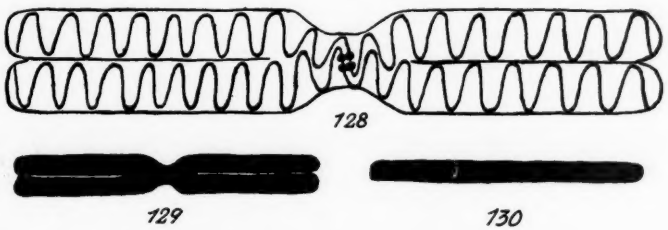
do not show any tendency to kinetic constrictions at first metaphase, but in a low frequency of chromosomes there are indications of such constrictions. During the second division the chromosomes are fairly small and it is difficult to make exact observations. There are certainly indications of kinetic constrictions to be seen, but they are not so clear as expected. During anaphase and also during metaphase of the second division the chromosomes are, however, strongly V-bent, which demonstrates that the kinetochore region has a low resistance to bending. The bending of the univalents in the first division is weak and of U-type. During first anaphase the chromosomes are only weakly bent. The tendencies to V-bend that are found in first anaphase chromosomes are most probably relics from the great mechanical deformation that is found in the ring bivalents at metaphase (of the type illustrated by the models Figs. 13—14). The same may be the case with other observations of tendencies to a V-bend in first anaphase chromosomes (*Lilium* and *Trillium*); so such bends cannot be used as arguments for a kinetic constriction in the first division. It should also be noted that a V-bend will arise even in a completely isopycnotic chromosome if only the pulling force is sufficiently strong or the resistance offered by the medium, in which the movements take place, sufficiently high.

kinetic constrictions. — Figs. 79—81. *L. tigrinum*. Second metaphase chromosomes in side view (Figs. 79—80) and polar view (Fig. 81) showing kinetic constrictions. — Figs. 82—95. First metaphase univalents (Figs. 82—94) and a rod bivalent (Fig. 95) in *Allium Cepa* × *fistulosum*. The kinetic constriction is clearly visible when the chromosomes are seen in the view where the two chromatids are lying side by side (Figs. 90—94), but it is not visible or scarcely so in the other figures where the two chromatids are lying upon one another (interpretation: see Figs. 128—130). — Figs. 96—105. Supercontracted root tip chromosomes of *Allium Cepa* showing distinct kinetic constrictions. — Figs. 96—98. Roots treated with saturated adaline for 12 hours. — Figs. 99—102. Roots treated with 0,1 mol pyramidone for 8 hours. — Figs. 103—105. Roots treated with saturated acenaphthene for 24 hours. — Figs. 106—116. *Paeonia*. — Figs. 106—109. First metaphase univalents (Figs. 106—108) and a bivalent (Fig. 109) in *P. anomala* × *tenuifolia*. — Figs. 110—114. First metaphase univalents in *P. officinalis*. — Figs. 106—114 show only weak kinetic constrictions except when the chromosomes are seen in the view showing the two chromatids side by side (Figs. 106 and 109). — Figs. 115—116. Second division in *P. officinalis*. — Fig. 115. Second metaphase chromosome in polar view showing a not very distinct kinetic constriction. Fig. 116. Second anaphase chromosome bent in V-shape. — Figs. 117—127. *Tulipa Gesneriana*. First metaphase chromosomes showing very distinct kinetic constrictions. — Figs. 117—126. Univalents. — Fig. 127. A trivalent (two kinetochores in focus). — All material fixed in chrome-acetic-formalin, in the case of Figs. 82—95 and 106—116 after prefixing in acetic alcohol. Microtome sections (with the exception of Figs. 103—105, which are from squash preparations). — Figs. 69—105 and 110—127 stained with crystal violet. — Figs. 106—109. Acetocarmine + crystal violet. — All figures × 1500.

In *Allium Cepa* × *fistulosum* and in *Paeonia anomala* × *tenuifolia* and *P. officinalis* the chromosomes show a distinct, although weak, kinetic constriction during first metaphase also. As expected from the presence of this constriction, the univalents in these materials also show distinct tendencies to a V-shape during first metaphase (compare the *Allium* univalents, Figs. 82—94, with those from *Secale*, Figs. 51—57). I have not studied the *Allium* chromosomes during the second division. In *Paeonia* the second metaphase chromosomes show a primary constriction (Fig. 115), which is not very distinct, and at second anaphase they are strongly V-bent (Fig. 116).

In *Tulipa* the kinetic constrictions are much greater than in any of the other materials during the first division. Thus, it may be said that the tulip chromosomes are approaching ordinary mitotic chromosomes in their structure during this stage. This similarity to mitotic chromosomes is actually manifested in the *behaviour* of the tulip univalents during first metaphase. In the other materials studied in the present paper the univalents are distributed at random over the spindle during first metaphase, but in the tulip cells the univalents show a strong tendency to be arranged on the equatorial plate. This fact gives definite support to the opinion advanced in the present paper, *viz.* that the difference in the degree of expression of the kinetic constriction is of fundamental importance in explaining the different chromosome behaviour found between ordinary mitosis and the first division of meiosis.

The univalents in *Allium Cepa* × *fistulosum* show a distinct kinetic constriction only when seen in that view where they show the two chromatids lying side by side (Figs. 90—94). When they are seen in the perpendicular view where the two chromatids are lying one upon the other no constriction is seen (Figs. 82—89). In this respect these meiotic chromosomes differ from supercontracted somatic chromosomes. In such somatic chromosomes a distinct constriction is visible in both these two views. These observations may indicate that the kinetochore region in these meiotic chromosomes is not really constricted, but that it differs from the chromosome arms merely by having a joint coiling of the two chromonemata, while there is a separate coiling of them in the chromosome arms (Figs. 128—130). If this interpretation is correct, there would not be any essential difference in kinetochore structure between *Allium* and *Tradescantia* during the first meiotic division. The chromosomes of *Paeonia* are very similar to those of *Allium*, but here there may actually be a slight constriction visible also



Figs. 128—130. An interpretation of the structure of the first metaphase chromosomes of *Allium Cepa* × *fistulosum* (a univalent is drawn). It is supposed that the chromatids have a joint spiral in the kinetochore region, but that they form individual spirals in the chromosome arms. — Fig. 128. Diagram showing the principle of coiling. — Fig. 129. The chromosome shows a constriction when seen in the view where the chromatids are lying side by side. — Fig. 130. No constriction is seen when the chromosome is observed in the view where the chromatids are lying one upon another.

in that view where the two chromatids are lying one upon the other. In such chromosomes as are assumed in the schemes of Figs. 128—130 the kinetochore region would represent a locus of mechanical weakness, and hence one would expect such chromosomes to tend towards a V-bend as observed in *Allium* univalents.

In *Tulipa* the kinetic constriction is very distinct in the first metaphase univalents in each of the two different views where the chromatids are lying side by side and where they are lying one upon the other.

In *Tradescantia* the two chromatids are coiled jointly during first metaphase, and they separate from one another to form separate spirals only at the transition from metaphase to anaphase (in connection with the so-called »lapse of chromatid attraction»). In *Secale*, *Triticum* and *Anthoxanthum*, too, the individual chromatids become visible only at the transition from metaphase to anaphase. In *Allium* and *Paeonia*, however, the individuality of the chromatids is invisible during early metaphase; during middle and late metaphase, on the other hand, the two chromatids form separate spirals lying side by side. This demonstrates that the lapse of chromatid association which initiates anaphase is a process that is distinct from the separation of the two chromatids from a joint spiral to form individual spirals. In spite of the individuality of the chromatid spirals they can remain associated with one another in some way or other.

The difficulties which I sometimes found in demonstrating di-

stinct kinetic constrictions during the second division of meiosis are a source of some dissatisfaction. One may well raise the question whether the kinetochores are of entirely »mitotic» type during this division. It may be possible that they are to some extent approaching the first division kinetochores in type, although still sufficiently »mitotic» to behave as in ordinary mitosis. This question should be subjected to special test in two ways: (1) by applying to the two meiotic divisions fixatives of a type which causes an exaggeration of the kinetic constrictions, such as, *e. g.*, Levitsky or certain osmic mixtures, and (2) by applying supercontracting reagents, such as are known to bring out the kinetic constrictions especially clearly in somatic mitosis.

There is a source of error which must be considered in a study of the kind made above, *viz.* that the meiotic chromosomes sometimes have *secondary* constrictions, which one must be cautious not to confuse with the kinetic constrictions. Fig. 45 demonstrates a rye chromosome having a secondary constriction of this kind. Similar constrictions were observed by PRAKKEN and MÜNTZING (1942, *e. g.* Fig. 121). The so-called »lump» chromosomes reported by MÜNTZING and PRAKKEN (1941) and by MÜNTZING and AKDIK (1948) are probably also examples of chromosomes having secondary constrictions. Similar chromosomes have been seen by the present writer in certain materials of *Anthoxanthum aristatum*. Fig. 46 is probably an example of another peculiarity that may be found in rye, *viz.* of the T-effect (*cf.* PRAKKEN and MÜNTZING, 1942; ÖSTERGREN and PRAKKEN, 1946). The chromosome ends of the bivalent in question are pointed and directed towards the poles. This effect is characteristic of certain inbred strains, but it may occasionally also be found in other rye plants. PRAKKEN (1943), for instance, reported it from some of his asynaptic plants of »Stålråg». Fig. 46 of the present paper is from another material, *viz.* from a partially asynaptic individual of »Östgöta Gråråg» containing supernumerary B chromosomes (*cf.* p. 149). The rye chromosomes paired in bivalents are usually strongly deformed in the regions between the kinetochores and the chiasmata because of the poleward pull working on the kinetochores. Even the univalents are not free from influence by these deforming forces; in extreme cases there may result a misdivision of the kinetochore at first anaphase (*cf.* below). The most distinct cases of kinetochore constriction in first metaphase chromosomes of rye are probably caused by such deforming forces. They would then represent constrictions induced by environmental influences working on the chromosomes rather than manifestations of their constitutional properties.

The reason why the whole of this study on the structure of the meiotic chromosomes was undertaken was, of course, to test the idea that during the first division of meiosis the chromosomes have a one-sided localization of the kinetic components. It is clear that when thick chromosomes show an isopycnosis of the kinetochore region, then there is no possibility for the small kinetochore apparatus to be localized on two opposite sides of the chromosome body.

There is also evidence of another kind for the unilateral localization of the kinetochore in the meiotic chromosomes. Thus, the kinetochore (or perhaps rather its »spindle spherules») is often elevated above the chromosome surface on the top of a small pin- or cone-like structure, a feature observed and described by many investigators (*e. g.*, SCHRADER, 1939). This has also been seen in the present study in many materials. For instance, the kinetochore is drawn out in this manner and distinctly visible in Figs. 15 and 17 from *Tradescantia.* Of course, there cannot be any doubt that this small kinetochore must occupy a unilateral position on these broad *Tradescantia* chromosomes. From this position it is elevated in the manner seen in the figures by the poleward attraction (the α-factor). In many of the other materials it could be seen in the bivalents at metaphase that the kinetochore was protruding above the chromosome surface on the top of a small cone. In the case of rod bivalents (and certain partners of trivalents) it could then be distinctly observed that this small cone was localized on one side of the chromosome body. This could be seen in *Secale, Triticum* (Fig. 59, the lower chromosome), *Triticale, Hyacinthus* (*e. g.*, Figs. 29 and 32), *Lilium* (Fig. 77, the upper chromosome), *Anthoxanthum, Paeonia, Allium*, and *Butomus* (Fig. 67). In the case of certain univalents as well it was possible to recognize the kinetochore as a small protrusion lying on one side of the chromosome body. This was observed in *Secale* and especially clearly in *Paeonia anomala* × *tenuifolia.*

Further evidence for the unilateral arrangement of the kinetochore is given by the way in which the spindle fibres attach to the univalents during first metaphase. It could be seen in rye and in the *Paeonia* hybrid studied that these fibres attach in a unilateral way on the kinetochore region of the univalents (*cf.* also below, p. 133, and Figs. 159—166).

It should be noted that in the case of the bivalents it could be seen that the kinetic sides of the two partner chromosomes were regularly oriented towards opposite spindle poles. The kinetochores of the univalents, however, were often oriented in various directions intermediate between the two opposite poleward ones during first metaphase.

The protrusion of the kinetochore on one side of the chromosome and the unilateral attachment of the spindle fibres were observations upon which LORBEER (1934) also founded his idea of a dorsiventral structure of the kinetochore region, its having a »Vorderseite» and a »Rückseite».

If we summarize the above results, we can conclude that there is

good evidence from many different materials that the chromosomes of the first meiotic division have a one-sided localization of the kinetic components, the kinetochore being localized on one side of the chromosome body. This characteristic of the meiotic kinetochore seems to be produced, mainly at least, by a different manner of coiling of the chromonema, which makes the kinetochore region isopycnotic (or practically so) during the first meiotic division. The most striking exception (*Tulipa*) to the rules described above actually gives further support to the present interpretations, as the univalents in this case simulate ordinary mitotic chromosomes in showing a strong tendency to arrange themselves on the equatorial plate.

DARLINGTON (1937 a) has coined a special term for the difference between the kinetochores of ordinary mitosis and those of the first division of meiosis, the difference which makes them auto-orient in mitosis but co-orient in meiosis. This difference was called the *polarization* [1] of the kinetochores. The term is a useful one and it may naturally be retained even though we can now see that the explanation of the phenomenon in question is different from what DARLINGTON supposed. According to DARLINGTON, the kinetochores of the first division of meiosis are completely undivided while the mitotic kinetochores are changed internally in a way that »is a preparation for division which is functionally equivalent to it». »This pre-division may be described as polarization. The centromere presumably becomes hour-glass shaped.» (*l. c.*, p. 539). At present it appears, however, that the essential difference between polarized and unpolarized kinetochores is a difference in the arrangement of the kinetic components relative to the chromosome body. In the polarized kinetochores (mitosis) the kinetic granules (spindle spherules or what we may like to call them) are lying on opposite sides of the chromosome (angular separation 180°), but in the unpolarized kinetochores (first meiotic division) they are arranged close to one another on one side of the chromosome (angular separation perhaps 10—20°). As described above, it would seem that this difference in arrangement may largely be due to differences in the coiling of the chromonemata. On the basis of the present interpretation it is well conceivable that there may also exist various intermediate degrees of polarization between the ordinary mitotic and meiotic types. In such cases the angular separation of the kinetic granules would have various values higher than the normal meiotic ones but smaller than

[1] The term polarization is used in chromosome studies to denote two other phenomena also (*cf.* DARLINGTON and MATHER, 1949, p. 409).

180°. Such cases may well be found in various apomictic organisms, where there occur types of nuclear divisions intermediate between mitosis and the first meiotic division. The case of *Tulipa* studied above seems also to belong to this group of more or less intermediate types.

In late metaphase — early anaphase it often occurs that lagging univalents start to behave like mitotic chromosomes, *i. e.* their kinetochores have become polarized. The mechanism of this special type of polarization is considered further below (pp. 115—117).

V. KINETOCHORE STRUCTURE AND BEHAVIOUR OF UNIVALENTS.

The structure suggested above of the kinetochore region at the first meiotic division can also shed some light on the characteristic *behaviour of unpaired chromosomes*. As is well-known, the univalents (in hybrids, haploids, triploids and asynaptic individuals) are usually distributed at random over the spindle during first metaphase. Naturally, the expression »random distribution» often used in this connection is only an approximate description. A quantitative analysis would certainly reveal in most (or all) cases that certain positions of the univalents are more preferred than the others; in some cases there is a more polar tendency, in others a more equatorial one.

From this so-called »random distribution» one might be tempted to suggest that the kinetochores of the univalents do not respond to the mitotic forces at metaphase, but that these chromosomes are passively distributed, more or less like acentric fragments (an idea expressed by SANCHEZ-MONGE, 1950). Some other authors have expressed similar ideas by saying that the response to the mitotic forces takes place only in pairs of units; for which reason the univalents with their »undivided» kinetic regions were supposed to behave in a different way from the bivalents. But these various interpretations are mistakes. *The univalents are not indifferent bodies* thrown around at random by cytoplasmic currents and other unspecific influences during metaphase. Their kinetochores respond actively to the mitotic forces in a way which would appear to be of essentially the same kind as in the paired chromosomes. This is evidenced by:

(1) The transverse equilibrium of the univalents. In the materials studied by me the univalents are usually lying on the surface of the spindle during metaphase, some of them close to the poles, others on the periphery of the metaphase plate and others, again, in various posi-

tions intermediate between the poles and the equator. The univalents are usually bent at the kinetochore region, and this region is directed inward towards the spindle axis, while the chromosome arms are directed outward. The bending of the univalents demonstrates that they are subjected to forces, and the chromosome arrangement in question is obviously a special case of the transverse equilibria discussed by ÖSTERGREN (1945 b). This arrangement suggests that the chromosome arms of the univalents are extruded from the spindle (the γ-factor of ÖSTERGREN, 1949 c) and that their kinetochores are attracted to the spindle poles, just as kinetochores in general are (the α-factor of ÖSTERGREN, 1949 c). This arrangement of the chromosomes was observed in the following materials: *Secale cereale, Triticum vulgare* × *turgidum, Triticum vulgare* × *Secale cereale, Anthoxanthum aristatum, Hyacinthus orientalis, Lilium candidum* × *chalcedonicum, L. tigrinum, Tulipa Gesneriana, Allium Cepa* × *fistulosum, Paeonia anomala* × *tenuifolia, P. officinalis.* (As already mentioned above, the *Tulipa* material differed by showing an unusually strong equatorial tendency of the univalents.)

(2) The second piece of evidence is supplied by a univalent *X* chromosome in the grasshopper study of KLINGSTEDT (1939), which was made on *Chorthippus bicolor* × *biguttulus.* In this material the *X* univalents generally have a random distribution over the spindle surface at metaphase, just as unpaired chromosomes in plants. In one cell in the hybrid grasshopper studied by KLINGSTEDT it had happened through a premeiotic accident that a chromosome arm of an *X* chromosome had been arrested in a cell wall. The *X* univalent remained with the end of its chromosome arm caught by the cell wall at metaphase, and it was found that the movements of this unpaired chromosome on the spindle had been so active that the chromosome was mechanically stretched in a similar manner as can otherwise be seen in bivalents where the two partners pull in opposite directions. Thus, the univalents can perform active movements on the spindle at metaphase, in spite of the absence of partners.

(3) A reference to the »transverse equilibrium» of the univalents has already been made above. The shape of the univalents in my materials gives, however, evidence of another kind, too, for the idea that their kinetochores respond actively to the mitotic forces. This is especially clear in *Paeonia anomala* × *tenuifolia,* but *Secale* shows indications of the same thing. Some univalents have a slightly attenuated kinetochore region and their arms are arranged in such a »lagging» manner,

as is well-known from anaphase chromosomes. The shape of these univalents suggests that their kinetochores are actively (and rapidly) moving in the longitudinal direction of the spindle (Figs. 156—159).

(4) Of some interest in the present connection is also the fact that the univalents can often be seen to be attached to the fibre structure of the spindle at metaphase (Figs. 159—166).

What is then the cause of the characteristic difference in arrangement that is found between the univalents and the paired chromosomes? It is natural to conclude that the difference is due to the absence of co-orientation in the unpaired chromosomes. We know that there must exist some kind of interaction between the two partners in each bivalent of such kind that their kinetochores come to be oriented towards opposite poles. (In the absence of an interaction the kinetochores would just as often have been oriented towards the same pole as towards opposite ones.) We may not know the mechanism by means of which this interaction takes place, but its existence is beyond doubt. And it is obvious that the univalents, which have no partners, cannot be influenced by this mechanism (at least not in the same way as the paired chromosomes). Thus, it is a natural conclusion that the different behaviour of paired and unpaired chromosomes is due to a *different orientation* of their kinetochores. In the paired chromosomes the kinetochores are regularly oriented towards the two opposite poles, but in the univalents they have probably a fairly random orientation in various directions. This idea is actually confirmed by direct observations (*cf.* above, p. 109).

Above there were presented certain reasons for the idea that at the first meiotic division the kinetochore region had four (or perhaps in some cases two) kinetic bodies (spindle spherules or centromeric chromomeres). These kinetic bodies were considered to lie on one side of the chromosome as demonstrated in Figs. 6—12. A kinetochore structure and arrangement of this kind is capable of explaining the characteristic behaviour of the univalents. It is possible on this basis to explain how a poleward attraction working on the kinetochore of a univalent (the a-factor) can result in an arrangement of this chromosome at the equator *as well as* at a spindle pole, and also in various positions intermediate between these.

A one-sided arrangement of the kinetic component as in anaphase of mitosis (Fig. 5) corresponds to a poleward movement, and a clearly two-sided arrangement (180° angular separation of the kinetic granules) corresponds to an equatorial equilibrium of the chromosome as

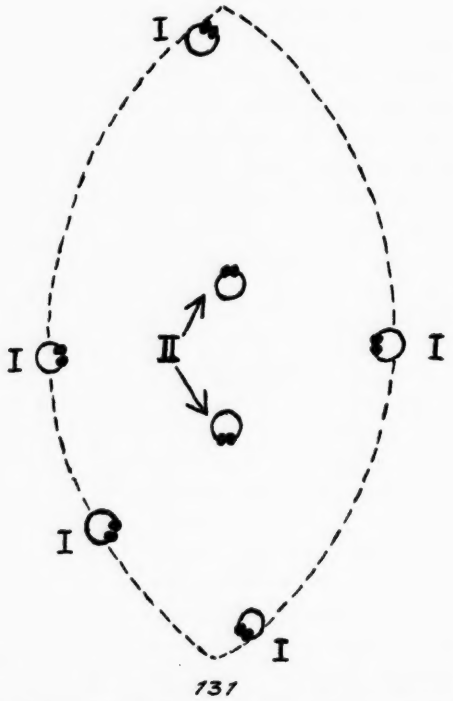


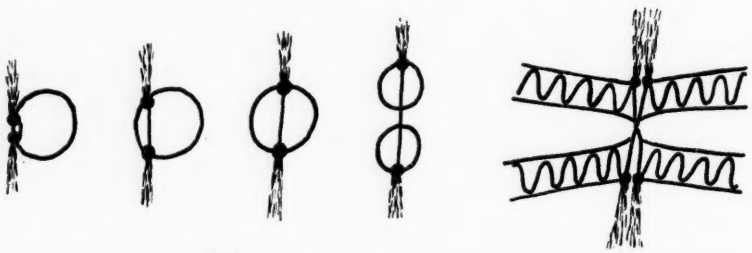
Fig. 131. Kinetochore orientation and the behaviour of univalents and bivalents. All the chromosomes are represented as cross sections through their kinetochore regions. The univalents are denoted by *I* and the two kinetochores which belong to a bivalent by *II*. When the univalents have their kinetochores oriented definitely towards the one pole or the other, they move to the pole in question, and when they are oriented about equally much towards both poles, then they take up an equatorial position. The two kinetochores of a bivalent orient themselves very definitely towards opposite poles (the co-oriented arrangement). The strong attraction of the kinetochores in the bivalent towards opposite poles results in an equatorial position of the bivalent.

in metaphase of mitosis (Fig. 4). As the univalents of the first meiotic division can take both these positions in the same cell, it is natural to conclude that their kinetochores are in a state intermediate between these two states. The one-sided arrangement of the kinetic bodies is not a perfect one (an angular separation of the kinetic granules somewhere between 0 and 180°). In this case the kinetochore is not obligatively restricted to be oriented with its kinetic granules towards one

pole only, but it has some possibility of also orienting in such a manner that its »spindle spherules» are exposed in the two opposite poleward directions (Fig. 131). In such a case the position of the univalent may depend upon the casual orientation of its kinetochore. If the kinetochore has happened to be oriented definitely towards one of the poles, then the α-factor will cause a movement of the univalent to this pole. But if the kinetochore is equally oriented towards both poles, then the α-factor will bring the chromosome to the equator (Fig. 131). Intermediate orientations may result in a movement of the univalents to various intermediate positions, and changes in kinetochore orientation may make the univalent dance to and fro on the spindle during metaphase (such movements were actually observed by BĚLAŘ, 1929, in the case of univalent *X* chromosomes in living grasshopper spermatocytes).

According to this interpretation, the univalents would have their kinetochores oriented in a random manner, while the two partners of each bivalent normally orient their kinetochores strictly in the two opposite poleward directions (Fig. 131). The origin of this difference in the kinetochore orientation between univalents and bivalents would actually constitute the main problem of the co-orientation. The forces acting on the kinetochores of univalents and bivalents (the α-factor) can be of quite the same kind and the kinetochore structure can also be the same both in paired and unpaired chromosomes (with the exception of secondary changes in structure that may develop through *deformation* of the kinetochores by the forces to which they are subjected; *cf.* below). *The different behaviour of univalents and bivalents at metaphase is explicable as resulting merely from differences in the orientation of their kinetochores.* The forces which bring about the arrangement of the univalents at random over the spindle and which also bring the bivalents into an equatorial equilibrium, these same forces also produce the anaphase movement, when the partners of the bivalents separate as a consequence of the lapse of chromatid attraction which initiates anaphase.

The kinetochores of the bivalents are subjected to strong forces pulling them towards opposite poles at metaphase. These forces are demonstrable from the fact that they stretch the chromosome regions between the kinetochores and the chiasmata, and the kinetochore regions are also mechanically deformed themselves by these forces. The »spindle spherules» are lifted above the chromosome surface and they are connected to the rest of the chromosome by attenuated fibrils (Figs.



132 133

Figs. 132—133. Diagrams showing the mechanical deformation of the kinetochores of univalents, a deformation which leads to their »polarization» and in many cases to their division at first anaphase. — Fig. 132. A series of cross sections showing the progression of the deformation. The separating kinetic granules are connected by zone 3 of the kinetochore (*cf.* pp. 92—93 and 95), which becomes more and more attenuated by the pull working in the two opposite poleward directions. In the last cross section the two chromatids have individual spirals, which have separated from one another after the lapse of »chromatid attraction» (*i. e.* the figure is from the anaphase stage); the two daughter univalents are then lying side by side, only connected by the attenuated fibrous structure of zone 3. — Fig. 133. Side view corresponding to the last cross section of Fig. 132. — In all figures the chromosomal spindle fibres have been drawn.

12, 15 and 17), as already mentioned above. Concerning those univalents which are lying in a more or less equatorial region of the spindle and which thus have kinetic elements directed towards both poles, it is then natural to assume that the α-factor has a similar deforming influence on their kinetochores, too. But the kinetochore part, which in this case is subjected to deformation, would be zone 3, the interchromomeric connection between the two centromeric chromomeres. The result expected from a mechanical stretching in this way of zone 3 can be seen from Figs. 132—133.

The components that are separated in this deformation may be different in the case of different univalents. In some univalents the centromeric chromomeres belonging to different chromatids may be pulled towards opposite poles, an orientation corresponding to a »normal» longitudinal division of the kinetochore. In other cases it may occur that the centromeric chromomeres belonging to the same chromatid are pulled towards opposite poles, an orientation of the chromosome that eventually may lead to a transverse division of the kinetochore. It may also occur that three of the four kinetic granules are oriented towards the one pole and the fourth one towards the opposite pole. Details in the organization of the kinetochore region, differing from one material to another, will influence the frequency

of these various orientations. Such variable features of organization are: (1) The relative size of the longitudinal and the transverse gaps which separate the four kinetic granules from one another. (2) Single or double coil in the chromosome at meiotic metaphase, and also separate or joint spiralization of the two chromatids. (3) Variations in the degree of constriction of the kinetochore region. (4) There can certainly also be other features, which at present are unknown.

In any event, all these orientations of the kinetic granules may lead to an attenuation of zone 3 of the kinetochore, as illustrated in Fig. 132. We can expect that the kinetic chromomeres pulled towards opposite poles will gradually separate more and more, until they may finally be completely oriented in the two opposite poleward directions. Through this attenuation of zone 3 the kinetochores of the univalents would in their structure approach the kinetochores of chromosomes at ordinary mitotic metaphase. The consequence of this change will be that many of the univalents gradually approach the equatorial plane at late metaphase or early anaphase; and such an effect has indeed been reported by many investigators. After the lapse of chromatid attraction, which takes place at the onset of anaphase, the two chromatids of such a univalent have separate spirals, and each of them has a separate matrix. At this stage the two chromatids will be associated only through region 3 of the kinetochore (Figs. 132—133) and the tension in this region will be especially strong. This tension may lead to a precocious separation of the closely paired components of zone 3, which correspond to the two sister chromatids (as probably takes place in the longitudinal division of the kinetochores of lagging anaphase univalents). The tension leading to the misdivision will scarcely be intensified through the lapse of chromatid attraction, but it will still increase gradually and may ultimately lead to a breakage of the connection. A longitudinal (»normal») division of the kinetochores of lagging univalents is of very common occurrence and has been reported from many materials by numerous investigators. There are also many reports to be found in the literature of transverse division (misdivision) of the kinetochores (*e. g.*, DARLINGTON, 1939 b; PRAKKEN, 1943; ANDERSSON, 1947; SANCHEZ-MONGE, 1950).

Thus, the univalents would not divide actively when they are lagging at the first meiotic anaphase, not even when they divide in the »normal» longitudinal direction, but they would simply be pulled asunder by the α-factor before their kinetochore is ripe for a normal division. The kinetochores will most probably be ripe for an active

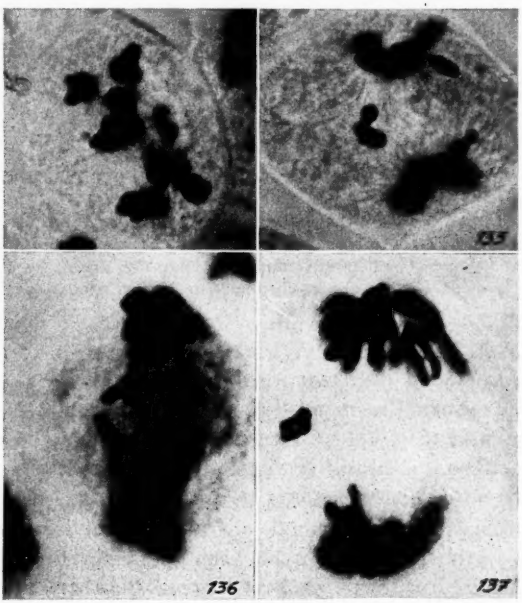
division only at the onset of the second meiotic anaphase. A misdivision through the tearing apart of the two centromeric chromomeres by the α-factor can naturally also take place in daughter univalents lagging at the second meiotic anaphase, as observed by SANCHEZ-MONGE (1950) and also interpreted by him in this way. It deserves to be mentioned that SANCHEZ-MONGE had already interpreted the first anaphase misdivision, too, as resulting from a tearing asunder of the interchromomeric connection by the α-factor. (The push-misdivision of SANCHEZ-MONGE would be a more unspecific variety of misdivision. It need not be discussed here, as the present paper is not intended to give a complete review of the misdivision.)

There are a number of observations that support the above interpretations. Thus, the following characteristic features of univalent behaviour should be noticed:

(1) The well-known fact that univalents lying in an *equatorial* position *divide* during the first anaphase of meiosis in many organisms, but univalents included in the *polar* groups in the same cells *do not divide*. This is caused by the fact that univalents are brought into an equatorial position by attraction towards both the opposite poles, and it is this same attraction which ultimately breaks the association inside the kinetochore and thus produces the division.

(2) In some cases it is possible to see that there is some component in the kinetochore region which offers resistance to the poleward pull; the daughter univalents are pulled apart a short distance but there is an *attenuated string* between them that prevents their separation (Fig. 134). Even in materials where the univalents divide, the daughter chromosomes often rest for a while side by side before the division is completed (*e. g.* in rye). And in some cases the attenuated string altogether prevents the separation, as in the B chromosomes of *Anthoxanthum aristatum* (but not in the A chromosomes) studied by ÖSTERGREN (1947 b).

(3) The division of the lagging univalents does not take place simultaneously in a cell (Figs. 135—136), but the separation of the daughter chromosomes occurs individually *in one univalent after another*; no doubt depending on the different time that will pass before the poleward pull has broken the association in region 3 of the kinetochore (*Anthoxanthum*, wheat, rye; personal observations). This fact is also of importance in demonstrating that the anaphase movement is not a concern for the cell as a whole, but that it is actually a



Figs. 134—137. Photographs of kinetochore behaviour in lagging univalents (the chromosome in Fig. 137 may, however, have been paired, as it is well-known that paired chromosomes in triploid hyacinths often behave like univalents; *cf.*, *e. g.*, DARLINGTON, 1936 a, p. 295). — Figs. 134—135. *Secale cereale*, a partially asynaptic plant from »Östgöta Gråråg». — Fig. 136. *Anthoxanthum aristatum*, triploid. — Fig. 137. *Hyacinthus orientalis*, triploid. — Fig. 134. A fibrous structure in the kinetochore region connects the separating daughter univalents. — Figs. 135—136. Asynchronous division of lagging univalents. — Fig. 137. The daughter univalents often remain associated side by side for a while before they separate. — Figs. 134—137. Fixation in chrome-acetic-formalin after prefixation in acetic alcohol. Microtome sections. — Figs. 134—135 and 137. Stain: Crystal violet. — Fig. 136. Stain: Aceto-carmine. — Figs. 134, 135, 137. × 1500. — Fig. 136. × 2000.

separate process for each individual chromosome (a fact demonstrated also by other data).

(4) Chromosomes that have been paired can also divide at their kinetochores during first anaphase. This may depend on the following two circumstances: (A) The chromosome has, although paired, failed to orient its kinetochore normally, as occasionally may happen, and it then behaves just as a univalent (failure of co-orientation; *cf.* DARLINGTON, 1937 a; see also below). (B) A kinetochore may be arrested during the anaphase movement by a dicentric chromosome

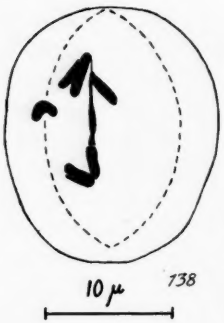


Fig. 138. *Allium Cepa* × *fistulosum*. A drawing showing chromosomes arrested by an inversion bridge. The unicentric chromatid is separating from the dicentric one in the upper chromosome. They are connected by an attenuated fibre in the kinetochore region. — × 1950.

bridge. The poleward pull may then break the connection inside the kinetochore between the normally unicentric chromatid and the dicentric chromatid (Fig. 138). Before the connection is broken, it often becomes an attenuated fibre (GEITLER, 1937, 1938 b; RICHARDSON, 1936; UPCOTT, 1937 a and b; RIBBANDS, 1937; DARLINGTON, 1937 b, 1939 b).

(5) In cases of misdivision (*i. e.* transverse division) of the kinetochore the fibrous connections between the separating parts form a very conspicuous feature (*e. g.* DARLINGTON, 1939 b). This is naturally in good agreement with the above interpretations.

(6) When different materials are compared there is found a certain independence between the tendency to equatorial distribution of the univalents and their anaphase division (*e. g.* KIHARA, 1931; hybrids in the wheat group). This is due to the fact that the component which determines the mobility (zone 2, the kinetic granules) is a different one from that which determines the division (zone 3). Changes in one of these components may take place independently of changes in the other one.

VI. CO-ORIENTATION AND PAIRING.

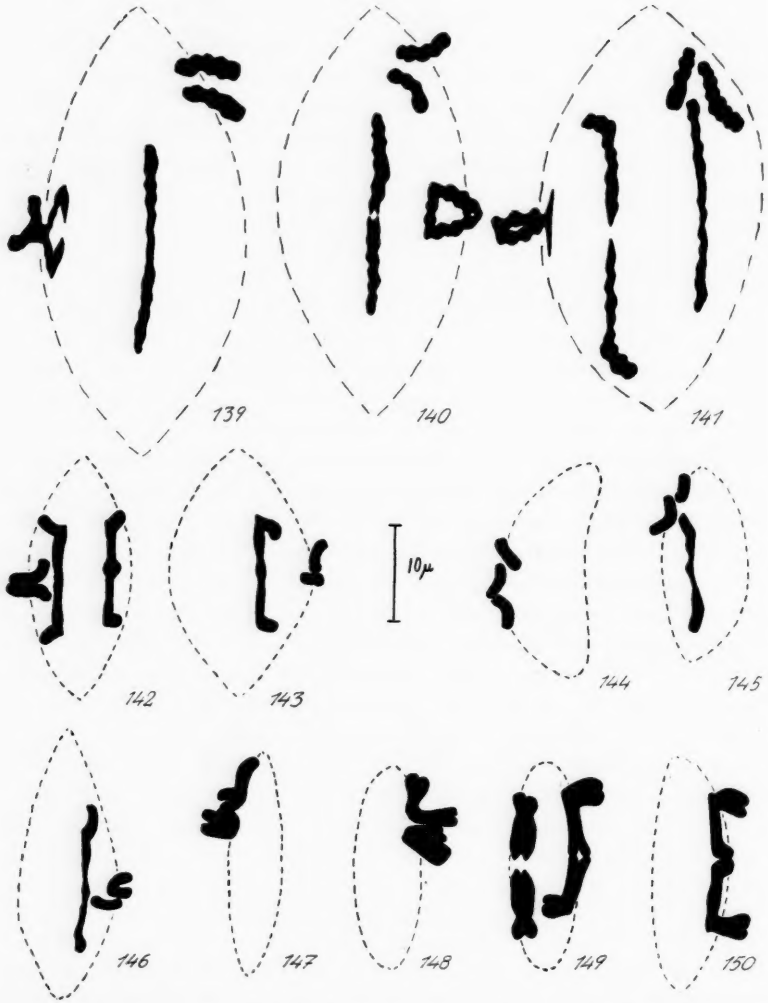
As already pointed out above (p. 89), it is a necessary conclusion that the phenomena of co-orientation must be due to some kind of interaction working between the kinetochores of the chromosomes involved in a bivalent or multivalent. According to the ideas of the present writer, the main factor governing the behaviour of the chromosomes on the spindle is an attraction of the kinetochores to the spindle poles (the α-factor). It may at first seem difficult, however, to see how such a poleward attraction would be able to bring about

the necessary interaction between the kinetochores, and for this reason one might feel tempted to postulate the existence of some additional factor that might account for this kinetochore interaction, *e. g.* a repulsion acting between the kinetochores.

The idea of a repulsion working between the kinetochores is not a new one. Many authors have suggested that repulsions are of great importance for the behaviour of the chromosomes during mitosis (see, *e. g.*, the review by SCHRADER, 1944). Such repulsions form an important part of the well-known theories developed by DARLINGTON (1936 a, 1937 a, 1939 a). DARLINGTON tried also to explain the lengthwise orientation of the bivalents in the spindle as resulting from such repulsions (of an electrical kind) in combination with the anisotropy of the spindle. These ideas cannot be maintained (see, *e. g.*, ÖSTERGREN, 1950 a), but in spite of this one might still consider it possible to use the idea of kinetochore repulsions (of some kind or other) as a *subsidiary factor* in order to explain the co-orientation. The behaviour of the chromosomes during the first division of meiosis *does not support* such an opinion, however.

If the co-orientation were due to a kinetochore repulsion, then this repulsion must be of a kind capable of working over quite long distances on the spindle. For it is known that even in the case of extremely long rod bivalents, where the kinetochores are very widely separated (75 % of the spindle length[1], for instance), the bivalents are still able to co-orient normally. The bivalents in a metaphase cell may, however, often be arranged quite close to one another, so that there is no repulsion or only a very small one working in the transverse directions of the spindle. So, if there were a repulsion, it would appear to work much stronger in the longitudinal than in the transverse directions of the spindle (as suggested by DARLINGTON). A study of the *univalents* demonstrates, however, that there cannot be any noticeable action of this kind working between their kinetochores, either in the transverse or in the longitudinal direction of the spindle. Thus, one can often find in materials having unpaired chromosomes that two univalents are lying with their kinetochores close together at metaphase and that the line passing through their kinetochores is parallel to the spindle fibres (Figs. 139—150). They are often much closer together than the partner kinetochores of the bivalents in the same cell. Such an arrangement of the univalents occurs, as far as can be judged without quantitative investigations, neither more often nor

[1] Personal observations in the newt *Triturus vulgaris*.



Figs. 139—150. Univalents and bivalents at first metaphase. There is an interaction between the kinetochores of such a kind that a normal co-orientation is possible even in long rod bivalents, but there is no repulsion between the kinetochores of univalents, even though these may lie close to one another and in such an arrangement that their connecting line is parallel to the fibre structure of the spindle. — Figs. 139—141. *Lilium candidum* × *chalcedonicum*. — Figs. 142—145. *Secale cereale*, partially asynaptic plants. — Figs. 142—143. From the material of PRAKKEN (1943). — Figs. 144—145. From a plant of »Östgöta Gråråg» (*cf.* p. 149). — Fig. 146.

more rarely than expected from randomness. A quantitative treatment of the subject would obviously meet with great difficulties and is scarcely necessary. I have studied this phenomenon in the following materials: *Lilium candidum* × *chalcedonicum, Secale cereale, Triticum vulgare* × *Secale cereale, Paeonia anomala* × *tenuifolia.*

The interaction which undoubtedly exists between the kinetochores of the bivalents leads to the result that the two kinetochores of each bivalent are pulled by strong forces in the two opposite poleward directions. If there had been an interaction between the kinetochores of univalents of the same kind, such an arrangement of their kinetochores close to one another as just described would never have been able to arise or at least it would not have continued until metaphase. Such cases were observed even in late metaphase cells.

It might be argued that the absence of an interaction in such pairs of univalents may be due to the fact that their kinetochores perhaps do not lie on a line that is absolutely parallel to the spindle fibres. It should be noted, however, that the interaction which is actually found between paired chromosomes is of such nature that there result forces pulling the partners towards opposite poles even in such cases where the connecting lines between the kinetochores are not parallel to the spindle fibres. This can clearly be seen from the behaviour of trivalents and other multivalents. It must also be considered that before the bivalents become co-oriented they lie in a random orientation, and consequently the interaction between the kinetochores which produces the co-orientation must definitely be capable of also working in cases when the bivalents lie oriented at big angles from the spindle axis.

Whatever the nature may be of the interaction working between the kinetochores of bivalents and multivalents, one thing is clear: this interaction does not work between the kinetochores of univalents even though they may lie closely approached to one another. The obvious conclusion is that this strange *kinetochore interaction* is of such nature that it *is dependent on the material association of the partners* (by means of chiasmata or even without chiasmata, *viz.* in organisms having chromosome pairing without chiasmata).

As will appear from the discussion further below, the poleward attraction of the kinetochores (the α-factor of ÖSTERGREN, 1949 c) gives

Triticum vulgare × *Secale cereale.* — Figs. 147—150. *Paeonia anomala* × *tenuifolia.* — × 1450.

an easy explanation of this kinetochore interaction which is working through the material association of the partners, and which produces the co-orientation.

Even if a kinetochore repulsion does not play an important rôle in the co-orientation of the bivalents and multivalents, it may naturally be possible that there still exists such an action and this may then play some rôle in certain other phenomena. Thus, it has been suggested that a kinetochore repulsion has an influence on the shape of the bivalents in the meiotic prophase and that it produces the terminalization movements of the chiasmata. My own studies of the meiotic prophase have, however, failed to demonstrate such an influence on the chromosome shape, and probably the terminalization movements may be given other interpretations, too. These questions will be discussed in more detail elsewhere. In the absence of more definite evidence for a kinetochore repulsion it appears as the most probable interpretation that there does not exist an action of this kind at any stage of mitosis and meiosis. As is well-known, there are at metaphase strong forces working on the partner kinetochores of the bivalents in the two opposite poleward directions of the spindle. These forces are manifested as a strong mechanical deformation of the bivalents. Of course, these forces need not in any way be due to a kinetochore repulsion. They are most probably just a manifestation of the attraction of the two partner kinetochores to the opposite spindle poles (the α-factor). There is a very striking change in the shape of the bivalents from diakinesis to first metaphase. This change ist most probably due simply to the fact that the bivalents come under the influence of the α-factor. There is no reason to introduce other assumptions.

VII. THE MECHANISM OF CO-ORIENTATION.

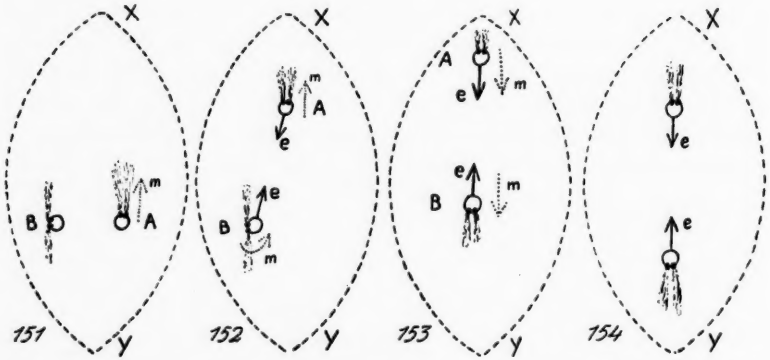
1. THE HYPOTHESIS OF ORIENTATION BY PULLING.

There is no reason to assume any differences in the constitution of the kinetochores between paired and unpaired chromosomes when they meet the spindle during prometaphase. On the contrary, it seems most improbable that the kinetochores lying in the same prophase nucleus and in the same physiological environment would take a different course of development dependent on whether they are lying in paired chromosomes or in univalents. It is true that one might suggest some kind of *position effect* working between kinetochores closely approached to one another in chromosome pairs, *e. g.* during pachytene. There can, however, be obtained evidence against a position effect of this kind from the behaviour of the chromosomes in polyploids and structural hybrids. In the case of many multivalents the kinetochores behave in the manner characteristic of paired chromosomes, even though they have not been closely associated with the kinetochores of the partner chromosomes during the meiotic prophase.

It must be the most probable assumption to believe that the kinetochores of paired and unpaired chromosomes have the same constitution and behave in the same manner when they first meet the spindle. The »random distribution» of the univalents over the spindle so often reported from metaphase studies is most probably due to a random orientation of their kinetochores in combination with a poleward attraction, as suggested above (pp. 111—115). It is to be expected that the univalents will change their positions as a result of changes in their kinetochore orientation; so it is probable that they are dancing around on the spindle in a more or less haphazard way during metaphase. Such movements were actually observed by BĚLAŘ (1929), who studied the behaviour of unpaired *X* chromosomes in living grasshopper spermatocytes (*Chorthippus lineatus*). He suggested that the movements were due to the univalents being passively pushed around by cytoplasmic currents, but according to the present interpretation the movement would be an active movement produced by the α-factor working on the kinetochores. This opinion of an active movement is proved by the observations of KLINGSTEDT (1939), who worked on a related grasshopper material (the hybrid *Chorthippus bicolor* $\times$ *biguttulus*). He found that an *X* univalent which had been caught by a cell wall during pre-meiotic irregularities became much attenuated during first metaphase of meiosis by the active kinetochore movements.

It seems most probable that the paired chromosomes behave in the same manner as the univalents when they first meet the spindle during prometaphase. They, too, start to dance around in a haphazard way on the spindle, depending on how their kinetochores change orientation. But, while the univalents can make these movements freely without interfering with one another, the paired chromosomes are greatly hampered in their movements by their mutual associations. The paired chromosomes will now and then come to exert a pull on one another. This effect should have very important consequences, as illustrated by Figs. 151—154.

Suppose, *e. g.*, that in a bivalent one of the two kinetochores (*A*) is oriented more definitely towards one of the poles (*X*) and that the other one (*B*) is lying in a more indifferent position (as will be the case in a more or less pronounced degree in practically all the bivalents on the basis of random orientation). Then, *A* will start to move towards the pole *X*, and after a while it will pull at the kinetochore *B* in the same direction. Now, *B* is attached by its kinetic side to the spindle, so



Figs. 151—154. The orientation-by-pulling hypothesis of co-orientation. Diagrammatic drawings of a bivalent during first prometaphase and metaphase. The bivalent is represented as cross-sections through its two kinetochores. In all the figures the chromosomal spindle fibres have been indicated. The two kinetochores have been denoted *A* and *B* and the two spindle poles *X* and *Y*. (Naturally these denotions are not intended to mean any qualitative differences, *A* is of the same kind as *B*, and *X* is of the same kind as *Y*.) The dotted arrows (*m*) denote *movements*, and the arrows (*e*) represent *elastic forces* arising from the extension of the chromosomes between the kinetochores. The forces pulling the kinetic granules towards the spindle poles (the α-factor) have not been represented in the figures. — Fig. 151. At first the two kinetochores meet the spindle in a random orientation. Under such circumstances the one or the other of them will in the majority of cases have happened to be oriented more definitely towards a pole than its partner is. In the present case *A* is fairly well oriented towards *X*. Consequently *A* will move towards *X*. — Fig. 152. When *A* has moved a certain distance, the chromosome parts connecting it to *B* become attenuated and give rise to elastic forces pulling at *B*. The kinetochore *B* will be dragged along by *A* towards the pole *X*. The kinetic granules of *B* are, however, attached to the fibre structure of the spindle. As they are attached, they will offer a stronger resistance to this dragging than the rest of the chromosome. For this reason the kinetic side of *B* will come to lag in this movement towards *X*. The result is a rotatory movement of *B*, its kinetic side becoming oriented more or less clearly towards *Y*. — Fig. 153. In its new orientation *B* starts to move towards *Y*, and it will drag *A* in the same direction. As a consequence *A* becomes more fully oriented towards *X*. — Fig. 154. The final result is the well-known metaphase equilibrium.

when *B* is moved by the pull of *A* the kinetic region of *B* will lag in the movement, and consequently it will be oriented in a direction opposite to the movement (Figs. 152—153). As a consequence of its changed orientation, which is now towards the pole (*Y*) opposite to that towards which *A* is oriented, the kinetochore *B* will start to move towards that pole (*Y*). Thereby it will exert a pull on *A*, which as a consequence of this pull will be more clearly oriented towards *X*. As a consequence of that, *A* will get an increased pulling force on *B*, *etc.* By pulling at one an-

other in this way, the kinetochores of the bivalents obtain an increasing degree of orientation towards the two opposite poles of the spindle, until finally both of them show a full poleward orientation (Fig. 154).

During this process now the one, now the other kinetochore will gain the upper hand in pulling at its partner, and hence it is to be expected that the bivalents will pendulate to and fro before they stabilize in the equatorial metaphase position. Such pendulations of the bivalents were actually reported by MICHEL (1943), who studied meiosis in living grasshopper spermatocytes (*Psophus stridulus*) that were photographed through a phase contrast microscope on a moving picture film.

The present hypothesis of the co-orientation may be called *the hypothesis of orientation by pulling*. It is based on the following points: (1) The kinetochore region is structurally differentiated with a special kinetic (or front) side. (2) The kinetochore is attracted towards that spindle pole towards which this side is oriented. (3) The kinetochores of the univalents and the bivalents attach themselves to the spindle. (4) It is supposed that the kinetochores can turn around and thus change their orientation in spite of the fact that they are attached to the spindle.

Point (1) must be regarded as definitely proved, there being evidence for it both from *chromosome structure* (*cf.* above) and from the *behaviour* of the chromosomes (ÖSTERGREN, 1950 a). The behaviour of the chromosomes also demonstrates the validity of point (2); *cf.* ÖSTERGREN (*l. c.*) and see also below. Point (3) is based on direct observations of the attachment of the fibrillar structure of the spindle to the kinetochore (*cf.* below). As regards point (4), there are observations demonstrating that kinetochores attached to the spindle are still able to turn around and change their orientation (PIZA, 1943; *cf.* also ÖSTERGREN, 1949 b, p. 466; HUGHES-SCHRADER, 1943 b, cited below on pp. 145—146; furthermore, I have made personal observations of this kind on plant meiosis, *cf.* below).

The mechanism of the chromosome movements. — It should be noted that the present hypothesis to explain the co-orientation is not based on any assumptions concerning the *origin* of the forces moving the chromosomes during mitosis. The four points upon which it is founded have an empirical derivation and they will exist independently of what hypothetical speculations may be developed in order to explain the origin of the mitotic forces. But, on the other hand, all hypotheses to explain the nature of the mitotic forces will have to pay due regard to these empirical results. The observation that the kinetochores are able to turn around in the spindle, in spite of

the fact that they are attached to its fibre structure, is a result which certainly gives the impression of being very closely related to the property of the chromosomal spindle fibres to allow a passage of bodies, such as the chromosomes, straight across them in a transverse direction (ÖSTERGREN, 1949 b). These results are in good agreement with the hypothesis of the spindle constitution and the origin of the mitotic forces as developed by ÖSTERGREN (1949 b). It should be noted, however, that the conclusions of the present paper are not based on this hypothesis, but that they have an independent existence.

Autonomous changes of kinetochore orientation. — The co-orientation is due to mutually correlated changes of orientation of the kinetochores involved in bivalents and multivalents. It is, however, most probable that there also exist cases of *autonomous* changes of kinetochore orientation, which take place in univalents and in paired chromosomes before their co-orientation. It would be very useful if the kinetochores, when they first met the spindle, started to dance around to and fro on the spindle in a haphazard way. Such a behaviour would highly facilitate the process of co-orientation by pulling to start, and it would render the whole mechanism much safer. BĚLAŘ's observations (1929) on univalent *X* chromosomes in living cells definitely support this idea of to-and-fro movements of kinetochores which have not co-oriented. Such movements suggest the existence of autonomous changes of kinetochore orientation, and the question concerning the mechanism of these changes arises immediately. It seems most probable that the *fourfold structure* of the kinetochore (Figs. 6—12) actually has the *adaptive purpose* of supplying a mechanism by means of which such irregular movements can be produced. Such a kinetochore can expose its kinetic granules in many different ways towards the two poles (4 : 0, 3 : 1, 2 : 2), and changes between these arrangements will be followed by corresponding changes of kinetochore position on the spindle. It is not quite clear how changes between these different orientations are produced; but a promising clue to the solution of this problem is given by the fact that when a kinetochore approaches one pole, then the forces working in that direction are weakened and those in the opposite direction strengthened (such are the properties of these attractions, as is demonstrated by other data). When the size of the forces working on the different kinetic granules changes in this way, then it is quite probable that a change of the orientation of the whole apparatus will result.

The orientation-by-pulling hypothesis gives a natural explanation for the fact that univalents do not behave like paired chromosomes even when they have happened to lie closely approached to one another. The interaction between the kinetochores found in the bivalents is working through the material association of the partners, as was concluded in an earlier paragraph. An interaction of the present kind is capable of also working between the kinetochores in very long rod bivalents in spite of the long distance separating them; an effect actually found at meiosis in many cases (*cf.* Figs. 139—150). Such long bivalents certainly form a serious obstacle to any effort to explain the co-orientation by means of repulsions, *e. g.* of electric type. How would it be possible to explain that the repulsion works over such a long

distance in paired chromosomes, when the univalents in the same cell do not manifest any mutual repulsions?

The present hypothesis is also in good agreement with the events of the prometaphase, such as they are found to take place during meiosis. When the bivalents first meet the spindle, they are lying in a haphazard arrangement as assumed in the hypothesis. There follow a co-orientation of the bivalents and a movement of them to the equatorial plate, and it is an important feature in these events that they do not take place simultaneously in all the bivalents, but that there is a separate reaction of each bivalent individually; some of them are found to remain in a helter-skelter arrangement while others have co-oriented but are still lying outside the equator and others, again, have co-oriented and reached the equator. This is quite in accordance with the expectations inherent in the hypothesis; the kinetochores are originally oriented in a haphazard way, and the movement leading to the co-orientation will start in those chromosomes whose poleward orientation has happened to be more definite than in the others. And, as the movements of the partners are at first uncoordinated, there will also for this reason elapse a time that is of different duration in different bivalents before the movements result in an efficient pull of the kinetochores upon one another.

Such an asynchronous course of the prometaphase events (separate reaction of individual bivalents) has been found in all the materials studied by me, and it has been reported in many papers by other workers.

2. OBSERVATIONS.

The above interpretations are supported by a number of observations on the prometaphase events made both by the present writer and by other investigators (*e. g.*, HUGHES-SCHRADER, 1943 b, 1947).

The phenomenon of the *prometaphase stretch* studied by HUGHES-SCHRADER in mantids and phasmids (1943 b, 1947) is in good agreement with the present hypothesis. In these insects there is a very conspicuous separation of the kinetochores towards the two opposite spindle poles when the bivalents meet the spindle, a separation which produces a conspicuous stretch of the chromosome regions between the kinetochores. This reaction and the following movement to the equatorial plate take place autonomously in the individual bivalents; some continue to lie unoriented at a time when others have co-oriented and stretched but still lie outside the equator and when others, again, have

reached the equator. This stretch is obviously the consequence of a pull towards the two opposite poles working on the kinetochores, as suggested by HUGHES-SCHRADER, undoubtedly the same pull as assumed above in the hypothesis of the present paper. (An additional factor of minor importance may be the elongation of the spindle in this stage, as pointed out by HUGHES-SCHRADER.) The autonomous behaviour of the bivalents during the stretch effect is quite in agreement with the explanation given above. This »stretch» process probably takes place in all organisms showing a co-orientation of the kinetochores, and this in a manner which is the same in principle though differing in degree. Thus, I have seen it in the following materials: *Tradescantia virginiana, T. paludosa, Trillium erectum, Lilium candidum, L. candidum* × *chalcedonicum, Tulipa Gesneriana, Secale cereale, Triticum vulgare* × *Secale cereale* (amphidiploid), *Anthoxanthum aristatum, Paeonia officinalis, P. anomala* × *tenuifolia, Scorzonera hispanica.* The stretch is not so pronounced, however, in these plants (and probably in most other materials) as it is in the insects studied by HUGHES-SCHRADER, a fact which is simply explained, I think, by the low degree of contraction of the chromosomes of these mantids and phasmids when they meet the spindle. Because of their weak contraction these chromosomes are much less resistant to the pull of the kinetochores. The intrachromosomal mechanism of contraction continues to work during this process and ultimately it overcomes the poleward pull with, as a result, a *recontraction* of the attenuated chromosomes which brings the kinetochores again closer to one another, as described by HUGHES-SCHRADER. In the plant materials studied by me there is no noticeable recontraction of this kind of the chromosomes, probably because they had already reached their full contraction when they entered into prometaphase.

HUGHES-SCHRADER is, I believe, slightly mistaken in her interpretations (1947, p. 17): »The essential feature of the pre-metaphase stretch is a precocious orientation and movement of the kinetochores toward the division centers — an orientation not ordinarily made until metaphase, and a movement ordinarily restricted to anaphase.» Actually, such orientations and movements are not restricted to metaphase and anaphase, but are normal and ordinary phenomena of the prometaphase; so there is nothing »precocious» about them in this respect. The essential feature separating HUGHES-SCHRADER's materials from other organisms seems to be a delay in the chromosome contraction, making the chromosomes more sensitive to mechanical stretch-

ing when they first meet the spindle. (Or one might also state, as is actually the same thing, that the start of the whole prometaphase is precocious relative to the chromosome contraction.)

It is obvious that such a stretch can only affect the chromosome region between the kinetochores and the points where the chromosomes are attached to one another (whether these attachments are due to chiasmata or not is irrelevant in the present connection). HUGHES-SCHRADER (1943 a) had found that the stretch effect is absent in *Callimantis antillarum*, in which species the chromosomes remain associated all along their length — except for a localized separation at the kinetochore region — until the onset of anaphase. It seems a simple explanation to suggest that the spindle and kinetochore forces act in essentially the same manner in this mantid as in the others, and that the absence of the stretch is simply due to a firm association of the chromosomes close to the kinetochores. There cannot be much of a stretch when there is practically no chromosome region to be stretched.

The criticism made by HUGHES-SCHRADER (1947, p. 18) of the interpretation of the chromosome movements given by ÖSTERGREN (1945 a) must be due to a misunderstanding. My opinion concerning the importance of the change from doubleness to singleness of the kinetochore apparatus at the transition from metaphase to anaphase is not a »subsidiary hypothesis» as she believes, but it is a direct and logical *conclusion* from the primary hypothesis concerning the type of forces affecting the kinetochore on the spindle. The arguments suggested by HUGHES-SCHRADER against my ideas give, indeed, a *strong support for* these ideas. Certainly the two widely separated kinetochores to which she refers must represent a very good case of doubleness of the kinetochore apparatus, a doubleness that is not only functional but structural. HUGHES-SCHRADER's misunderstanding may be due to the somewhat concentrated form in which my hypothesis was described at that time.

I have also made some personal observations on the chromosome behaviour which support the present interpretations. In a study on the chromosome movements made on slides from fixed and stained materials it is naturally often difficult to judge whether or not the chromosomes were moving when the cell was fixed and also to decide in what direction the movement took place. The mechanical deformation of the chromosomes is, however, often a good tool in solving such questions.

All organisms are not equally favourable for use in such studies. It is clear that the mechanical deformations will usually be much more conspicuous in long and slender chromosomes than in strongly contracted ones. Thus, the very broad chromosomes of *Tradescantia* are but little deformed by the mitotic forces during the first meiotic divi-

sion, and therefore this material is not very good for the present purpose. The deformations also depend upon factors which can scarcely be predicted by an investigator, such as, *e. g.*, the relative amounts of (a) the velocity of the chromosome movements, (b) the softness of the chromosome bodies, and (c) the resistance offered by the surrounding medium (spindle and cytoplasm) to the chromosome movements.

When a study is undertaken of possible independent movements of the two kinetochores belonging to the same bivalent, it is very useful that the chromosome regions connecting the two kinetochores are very long so that the movements of each of them is registered separately by deformations of the chromosome body. Thus, it is not good to use such materials as have a great number of chiasmata distributed along the whole length of the bivalents, nor is it advisable to have materials with a proximal localization of the chiasmata. Such materials as *Hyacinthus*, *Lilium* and *Fritillaria* are consequently not very suitable for studies on the prometaphase mechanics. Some of the erroneous hypotheses suggested in this field may originate from the fact that their proponents were mainly acquainted with such materials.

It is advantageous to have materials with a distal localization of the chiasmata, and it is also useful when the chiasmata are reduced in frequency (*e. g.* by hybridity), as then the bivalents are simpler and easier to analyse. *Paeonia* was found to be an excellent material for the present purpose, and rye and rye-wheat amphidiploids could also afford some information. The hybrid *Lilium candidum* × *chalcedonicum* has a reduced frequency of chiasmata and often a distal localization of them, but it was found that the chromosome deformations were inconspicuous before the co-orientation; presumably the movements are too slow relative to the viscosity of the medium.

The questions to be elucidated were the following ones:

(1) Are the univalents attached to the spindle, as the paired chromosomes are known to be?

(2) Do the univalents perform active movements on the spindle during prometaphase and metaphase?

(3) Are the paired chromosomes moving actively before their co-orientation? Are these movements of the two kinetochores in a bivalent initially coordinated with one another, or are they random?

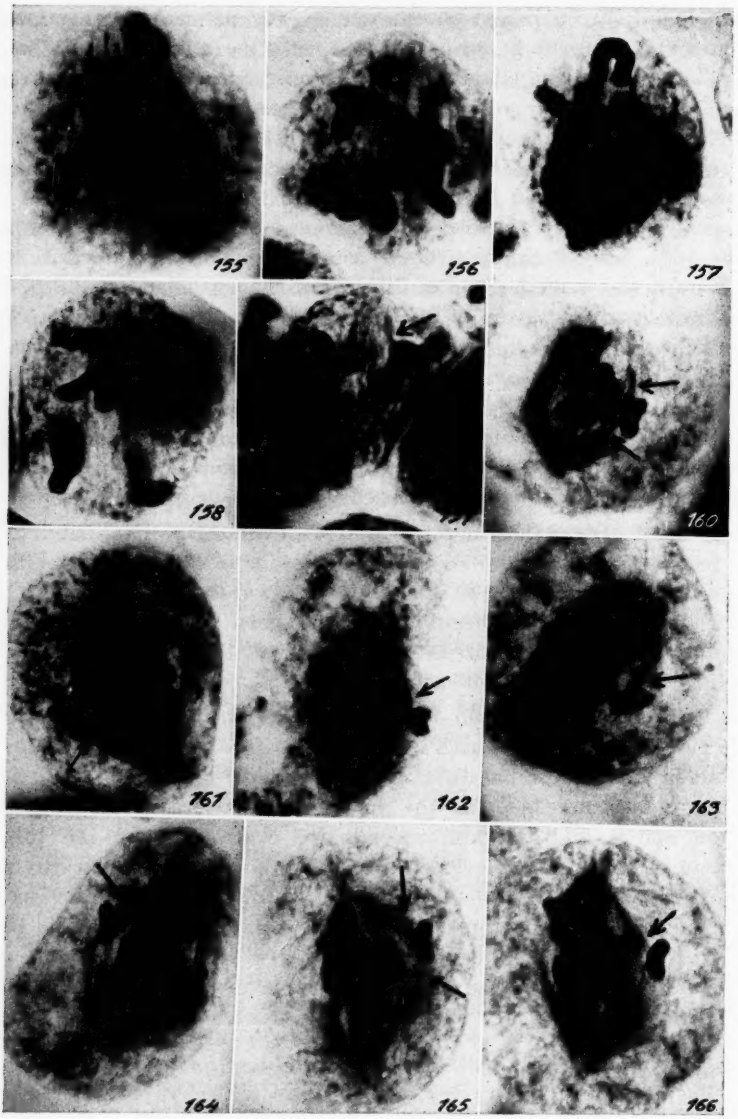
It is generally well-known that the kinetochores of the bivalents are furnished with chromosomal spindle fibres (*cf.*, *e. g.*, SCHRADER, 1944), but there has not been paid much attention to the univalents in this respect. As is well-known, there are profound differences in the

behaviour between paired chromosomes and univalents during metaphase, and it might be suspected that only the paired chromosomes were attached to the spindle, while the univalents behaved like acentric fragments (such an opinion was expressed by SANCHEZ-MONGE, 1950). As already mentioned above (pp. 111—113), there is evidence against this view (*cf.* KLINGSTEDT, 1939). And actually, a study of my material of *Secale* and *Paeonia* demonstrated that the kinetochores of the univalents are furnished with chromosomal spindle fibres (Figs. 159—166). Sometimes such fibres [1] can be seen directed towards one pole only, and sometimes towards both of them; sometimes the fibres (or fibre bundles) have a different thickness in the two opposite poleward directions, sometimes they seem to have the same. (A reference to these fibres of the univalents was made earlier by ÖSTERGREN and PRAKKEN, 1946, p. 486.) YASUI (1941), who studied hybrids of *Papaver*, observed also that the univalents were attached to chromosomal spindle fibres. She supposed that these acted as traction fibres on the univalents and produced their poleward movements.

An interesting fact in connection with these fibres is that it can be seen that they attach to a point on one side of the univalent, either they are directed to one of the poles or towards both of them. This fact gives further evidence for the conclusion presented above that the kinetochore is localized on one side of the chromosome body during the first meiotic division.

Especially in *Paeonia anomala* × *tenuifolia* (but sometimes in rye also) it can often be seen that the univalents perform active movements in the longitudinal direction of the spindle (Figs. 156—159). The chromosomes are bent in the manner characteristic of anaphase chromosomes and the kinetochores are drawn out by the forces pulling at them. The movement can be directed as well towards the nearer pole as to the more remote one. Obviously, the *direction* of the movement is not dependent on the distance from the one pole or the other. Hence, the observations agree with the idea that the kinetochores are attracted towards the pole towards which they are oriented, irrespective of whether this is the nearer one or not. Univalents

[1] It should perhaps be remarked that the expressions »fibres» and »fibre bundles» are used in the present paper in a purely descriptive way to denote these structures. It is quite possible (and even probable) that there are not any real continuous fibres connecting the kinetochores to the poles, but that these connections are rather of liquid crystalline or tactoid type (*cf.* ÖSTERGREN, 1949 b).

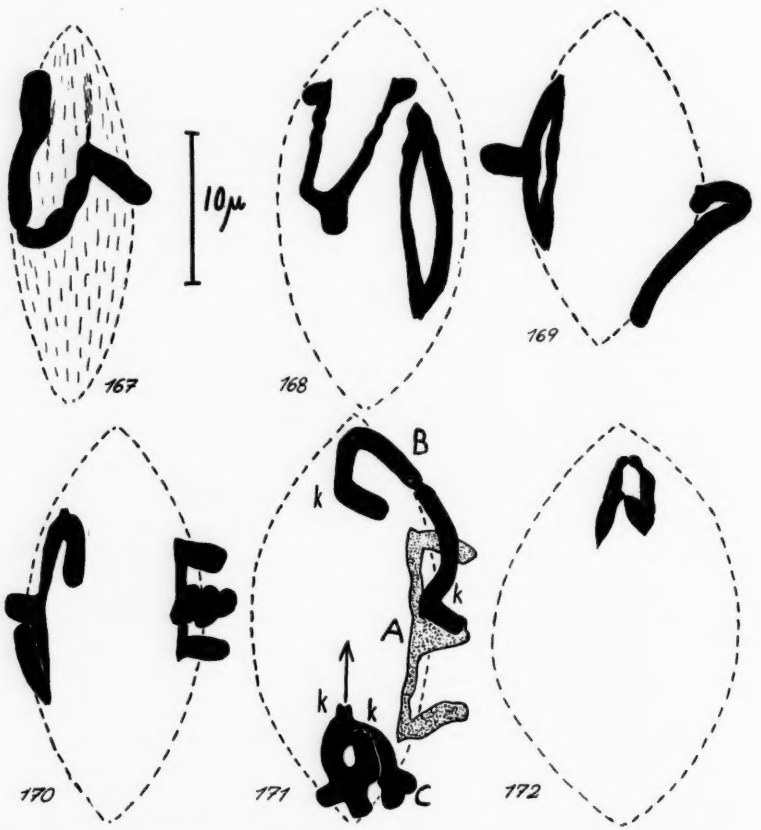


Figs. 155—166. Behaviour on the spindle of univalents. — Fig. 155. *Anthoxanthum aristatum*, triploid. The ordinary arrangement of univalents. They usually float on the surface of the spindle, some of them close to the poles, some close to the equator

suggesting such movements occur even in late metaphase, but they are especially common in prometaphase and early metaphase.

Conspicuous deformations of paired chromosomes of the kind expected from the present theory were found during prometaphase in *Paeonia officinalis, P. anomala* × *tenuifolia* and in amphidiploid *Triticum vulgare* × *Secale cereale* (Figs. 167—172 and 173—179). It was found that the kinetochores of the bivalents perform vigorous movements on the spindle even before the bivalents in question have co-oriented. The spindle forces draw out the kinetochores and cause conspicuous deformations of the chromosome bodies. The movements appear to be uncoordinated at first, as both the kinetochores of a bivalent can be found moving towards the same spindle pole. Such movements can take place both towards the nearer pole and towards the more distant one. This suggests that the direction of the movement is independent of the position of the kinetochores relative to the poles, but that it is decided, instead, by the orientation of the kinetochores. The orientation of the kinetochores in a bivalent is obviously at first a random one and both of them can be oriented towards the same pole. The paired chromosomes behave initially just like univalents on the spindle. These vigorously moving chromosomes are often lying well inside the spindle (thus, there is no justification for assuming a participation of cytoplasmic currents in these effects). In other bivalents the chromosomes seem to be more at rest during prometaphase — early metaphase. They can be found floating on the surface of the spindle, as univalents often are, with the kinetochores directed inward and with the chromosome bodies in the cytoplasm (Figs. 167, 169, 171, 174, 175). The kinetochore is attached to chromosomal spindle

and others in various intermediate positions. Three univalents are in focus in Fig. 155. — Figs. 156—159. *Paeonia anomala* × *tenuifolia.* Active movements in the longitudinal direction of the spindle made by univalents at metaphase. The deformations of the univalents clearly demonstrate such movements. In Fig. 157 the univalent has reached the pole, so the localized force at the kinetochore has disappeared and the chromosome is relaxing from the movement deformation; it is straightening and has changed from a V- to a U-shape. In Fig. 159 the chromosomal spindle fibres are visible (at the arrow). — Figs. 160—166. *Secale cereale*, a triploid plant (from an inbred line showing the T-effect; the material of PRAKKEN and MÜNTZING, 1942, and ÖSTERGREN and PRAKKEN, 1946). The figures show the attachment of chromosomal spindle fibres to univalents at metaphase (ordinary univalents, not T-univalents, are photographed). The chromosomal spindle fibres are marked by arrows. — All fixations made in chrome-acetic-formalin after prefixation in acetic alcohol. Microtome sections. — Figs. 155 and 160—166. Stained in acetocarmine. — Figs. 156—159. Double stained in acetocarmine and crystal violet. — Fig. 155 × 2000. — Figs. 156—166 × 1500.



Figs. 167—172. Drawings showing the prometaphase behaviour of bivalents. — Fig. 167. *Paeonia anomala* × *tenuifolia*. — Figs. 168—171. *Paeonia officinalis*. — Fig. 172. Amphidiploid *Triticum vulgare* × *Secale cereale*. — Fig. 167 (= the photograph Fig. 173). The left chromosome of the bivalent is floating in an »indifferent» manner on the spindle surface (as most univalents usually do at metaphase). The right chromosome is actively moving towards the upper pole. Its kinetochore is drawn out and its body deformed by the pulling force. — Fig. 168 (= the photograph Fig. 178). In the left bivalent both the kinetochores are actively moving towards the upper pole. Note the drawn-out kinetochores and the deformed chromosome bodies. The right bivalent is fully co-oriented and congressed. — Fig. 169. The left bivalent is fully co-oriented and congressed. The lower chromosome of the right bivalent is floating in an »indifferent» manner on the spindle surface. Probably the upper chromosome has just started to move. The kinetochore is drawn out. — Fig. 170. The right chromosome has a typical metaphase appearance. Its left neighbour shows by the arrangement of its arms that the two kinetochores have just moved towards the two opposite spindle poles (from an initially more equatorial

fibres (Figs. 167, 174) directed towards one or both of the poles. In these chromosomes the kinetochores probably have a more or less intermediate orientation, so that kinetic components are exposed in both the two poleward directions. The two kinetochores of such a bivalent can sometimes be found to lie on a line parallel to the fibres of the spindle, without manifesting any mutual repulsions (Figs. 174, 175). Such an arrangement would naturally be expected to occur now and then on the basis of the present theory; so there is nothing problematic in it. This arrangement, however, strongly disagrees with the interpretations of DARLINGTON (1936 a, 1937 a, 1939 a).

The following conclusions can be drawn from the observations:

(1) There is an active poleward attraction of the kinetochores during prometaphase, an attraction directed towards the pole towards which the kinetochore is oriented.

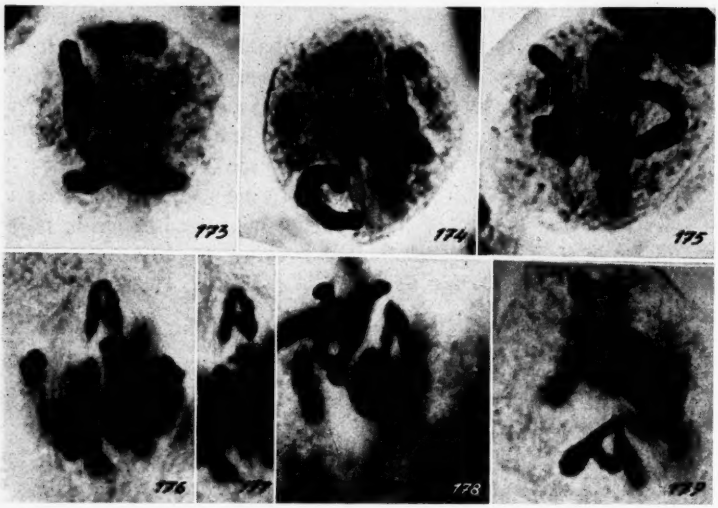
(2) The paired chromosomes behave at first like univalents when they meet the spindle. Their first movements are not mutually coordinated; the kinetochore orientation in the bivalents is initially at random.

(3) The kinetochores are capable of turning around in spite of being attached to the spindle.

(4) The chromosome behaviour goes definitely against the electrostatic hypothesis of DARLINGTON, which assumes repulsions separating the kinetochores from one another and pushing them from the poles towards the equator. The interpretations by BERNAL (1940, 1949) are also disproved as far as his »negative tactoids» are concerned. He assumed that the kinetochores of the bivalents were separated from one another by a »negative tactoid» forming between and around each pair of kinetochores. It was supposed that this negative tactoid, as a whole, moved into the equatorial region.

The observations give such support to the orientation-by-pulling

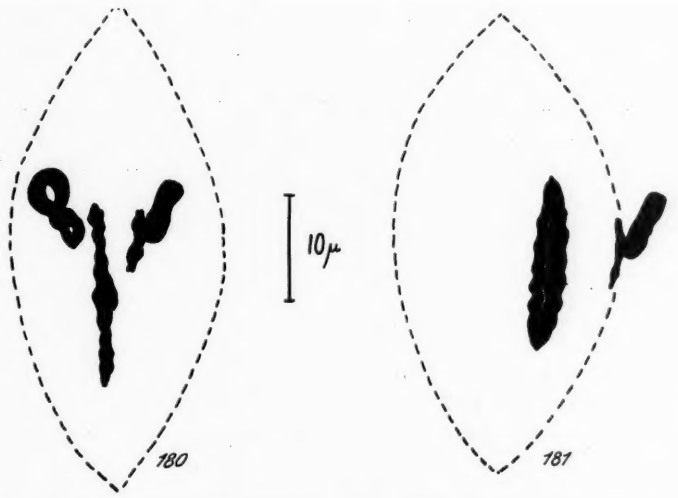
position). — Fig. 171. Bivalent *A* (which is dotted) is fully co-oriented and congressed. Bivalent *B* has both its chromosomes floating in an »indifferent» way on the spindle. Bivalent *C* shows active kinetochore movements towards the upper pole. (The direction of the movement indicated by an arrow.) In *B* and *C* the kinetochores are marked by *k*. — Fig. 172 (= the photographs Figs. 176—177). A ring bivalent in which both the kinetochores are moving actively towards the lower pole. The kinetochores are drawn out to points by the pulling forces and the chromosomes are bent by the movement. In the upper part of the bivalent two secondary constrictions are visible. — × 2300.



Figs. 173—179. Photographs showing the prometaphase behaviour of bivalents. — Figs. 173—175. *Paeonia anomala* × *tenuifolia*. — Figs. 176—177. Amphidiploid *Triticum vulgare* × *Secale cereale*. — Figs. 178—179. *Paeonia officinalis*. — Fig. 173. See Fig. 167. — Fig. 174. The two chromosomes of a bivalent are floating in an »indifferent» manner on the spindle surface. The upper chromosome has its kinetochore attached to chromosomal spindle fibres, which are visible in the figure. — Fig. 175. A ring bivalent floating on the spindle surface. The kinetochores and one terminal chiasma in focus. The chromosomes are bent at the kinetochores and the other half of the ring is behind the visible half. — Figs. 176—177. The bivalent of Fig. 172 photographed at two different focal levels. — Fig. 178. See Fig. 168. — Fig. 179. The bivalent in the lower part of the figure shows active movement of its upper kinetochore towards the upper pole. The lower kinetochore does not show evidence of active movement. — All material fixed in chrome-acetic-formalin after prefixation in acetic alcohol. Microtome sections. — Figs. 173—177. Acetocarmine and crystal violet (double stain). — Figs. 178—179. Crystal violet. — × 1500.

interpretation of the co-orientation that this interpretation may well be considered as proved.

During the later stages of prometaphase and during early metaphase one can often see *skew bivalents, i. e.* bivalents that have most of their chromosome body lying on one side of the equatorial plate, and which may also show an unequal tension on the two kinetochore regions (Figs. 180—181). The shape of these bivalents is easily understood as caused by a lagging of the chromosome body in the movement of the co-oriented bivalent towards the equator, in a similar way as



Figs. 180—181. *Lilium candidum* × *chalcedonicum*, prometaphase of the first division of meiosis. Two cells showing skew bivalents (besides normal ones). The deformation of the bivalents is presumably due to their movements in the longitudinal direction of the spindle. — × 1650.

the chromosome body lags in the anaphase movement. DARLINGTON (1936, p. 285 and Figs. 13—14, p. 286) has suggested that such bivalents were due to a »body repulsion» pushing the chromosome body out of the equatorial plate and away from the other chromosomes. I have observed, however, that the chromosomes often have this shape even when there is no other chromosome body in such a position as to be able to cause a repulsion of this kind. The skew bivalents are especially common in early metaphase (and naturally prometaphase), which suggests the present interpretation. I have also seen that the chromosomes (both bivalents and univalents) can lie closely approached to one another in these cells without showing signs of repulsion. Consequently, I think there is no reason to believe that there exists a body repulsion capable of causing such spectacular chromosome deformations. The only effect that DARLINGTON's electrostatic body repulsion may be suspected to produce is a reduction of the instances of direct contact between the chromosomes (and it may well be questioned whether such a repulsion exists at all at the cytological level of analysis).

It is probable that the bivalents do not arrange themselves in a stabilized equatorial position at once, but they may pendulate to and fro across the equator in the longitudinal direction of the spindle before they stabilize. Such a pendulation of the bivalents was observed by MICHEL (1943) in living grasshopper spermatocytes. During these pendulations there should also be good opportunities for the bivalents to become distorted to the skew shape observed here.

Such skew bivalents were often present in my slides of *Lilium candidum* × *chalcedonicum*, and they were also seen in *Secale*, *Paeonia*, and *Allium*.

3. OTHER PHENOMENA TO BE CONSIDERED HERE.

There are some other effects that must also be considered in the present connection, *viz.* the occasional cases of *failure* of the co-orientation mechanism to work and also the co-orientation of *multivalents*.

In the orientation-by-pulling hypothesis of co-orientation presented above it is assumed that the partners of the bivalents at first dance around at random over the spindle. In the course of these movements their kinetochores sooner or later come to exert a pull on one another by means of the chromosome regions connecting them, a pull which leads to their co-orientation. In some bivalents the two kinetochores are connected by only short chromosome regions, this being so because of the shortness of the paired chromosome arms or the localization of the chiasmata. In such bivalents the two kinetochores have only quite small possibilities of moving without pulling at one another; so it may be expected with a high degree of safety that they co-orient very soon. In other cases, however, the chromosome regions connecting the two kinetochores are very long relative to the spindle size, and in such bivalents the kinetochores should have very good possibilities of making extensive movements on the spindle without interfering with the movements of one another. Consequently, it is not surprising to find that some bivalents, especially such formed by long chromosomes, may sometimes *fail to co-orient*. Such a failure of co-orientation has been reported many times by various authors (*e. g.*, DARLINGTON, 1936 a, b, 1937 b, 1939 b; RICHARDSON, 1936; RIBBANDS, 1937; KLINGSTEDT, 1939). Some of the cases appearing like failure of co-orientation may actually be cases where the orientation is only delayed (as pointed out by DARLINGTON, 1937 b), but in other cases there is no doubt that anaphase starts without a normal orientation

of the bivalent in question and then one can speak of a real failure of orientation. In cases of failure of co-orientation the two partner chromosomes behave quite as two univalents on the spindle. The chromosomes can often be found to lag and divide at anaphase, just like ordinary univalents.

Also, it is quite in accordance with expectation to find such observations as that of HUGHES-SCHRADER (1947, p. 8), who reports that the biggest bivalent in *Isagoras subaquilis* (a phasmid) shows difficulties and is delayed at the co-orientation during prometaphase. She concluded that the chromosomes of this pair »seem to have approached the maximum size compatible with a smooth functioning of the spindle mechanism», a conclusion in complete agreement with the present ideas.

Accessory plates. — DARLINGTON (1936 a) has coined the term »accessory plate» to denote bivalents that have been found outside the equatorial plate during metaphase. These bivalents are either such as have failed to co-orient or such as have not yet reached the plate although they have co-oriented. The term is based on interpretations founded on DARLINGTON's electrostatic hypothesis of the chromosome movements, and at present it must be concluded that this expression »accessory plate» is worthless both as a descriptive term and as an explanation of the situation found in such cells. (The term has been used by many other investigators, and even as late as in 1950). DARLINGTON (1936 a, b) has also expressed the opinion that such a remaining of chromosomes outside the equatorial plate is due to a crowding of big chromosomes in a small spindle. This interpretation does not appear convincing at all. The α-factor which produces the movement to the plate is quite strong, judging from the deformation it causes of the bivalents. One might well expect it to be capable of producing a congression against even a considerable resistance. And what would produce the »resistance» in connection with a »crowding»? The chromosomes need only very little space in the spindle; the whole chromosome body is capable of lying in the cytoplasm, only the kinetochores must be localized in the spindle. The behaviour of the chromosomes definitely tells against the existence of both a mutual kinetochore repulsion and a body repulsion of any noticeable magnitude; so such effects are not likely to cause any interference. Those cases considered as due to crowding do not show the abnormal chromosomes being pressed against the others, which are already on the equator. There is, I think, every reason to consider such cases as due to a simple delay or failure of co-orientation.

The functional purpose of the metaphase stage. — Starting from the ideas developed in the present paper, it is possible to develop an opinion concerning the functional purpose of the metaphase stage. The organizational details of living organisms very often have a high degree of utility for the species carrying them, a fact that is a simple consequence of the interaction of natural selection with the variation produced by mutations and other genotypical changes. It is certainly to be expected concerning such a widespread feature, found in practically all organisms, as the metaphase stage, that it must also have a definite useful purpose to fulfil.

The metaphase stage is characterized by an arrangement of the chromosomes or chromosome configurations on a regular equatorial plate on the spindle. It must be pointed out that such an equatorial arrangement is by no means in itself a necessary condition that must be fulfilled if mitosis (meiosis) shall perform its primary purpose of distributing the daughter (partner) chromosomes in a regular manner to the two spindle poles. The only condition necessary for this end is that all the chromosomes have been regularly auto-oriented (in mitosis) or co-oriented (in the first division of meiosis). If such an orientation is followed by an anaphase separation, the purpose of the division is fulfilled, and this should theoretically be quite possible without the intercalation of a metaphase congression between the orientation stage and the anaphase movement. What is then the significance of the metaphase stage?

The purpose of the metaphase stage is understood if we consider the characteristics of the orientation stage. The time for the orientation of the individual bivalents is to a large extent dependent on randomness, a fact leading to a considerable asynchronism in their orientation. As a consequence of this, it must also be expected that there will be a great difference in the time necessary for the different cells to finish their orientation stage. It is obvious that the lapse of association of the partner chromosomes which initiates their anaphase separation must not take place before all the bivalents have performed a full co-orientation. As the duration of the orientation stage is quite variable between different cells, it is clear that it will be necessary for the onset of anaphase to be kept fairly late compared with the average time taken by the co-orientation, this just in order to get a sufficiently safe margin of time to insure that the delayed bivalents and cells have also co-oriented. A simple result of this margin of safety is that the bivalents in the great majority of cases will get time to settle down in the equatorial equilibrium known as the metaphase arrangement. The functional purpose of the forces that produce the metaphase equilibrium (the α-factor) is (*a*) to co-orient the bivalents, and (*b*) to produce the anaphase movement. Seen in this way, the metaphase arrangement appears as a phenomenon without a useful purpose of its own, resulting simply as a by-product of more purposeful features of the division, *viz.* as a result of the operation of the co-orientation forces during a safety margin of time devised to insure a complete co-orientation in all cells before anaphase starts.

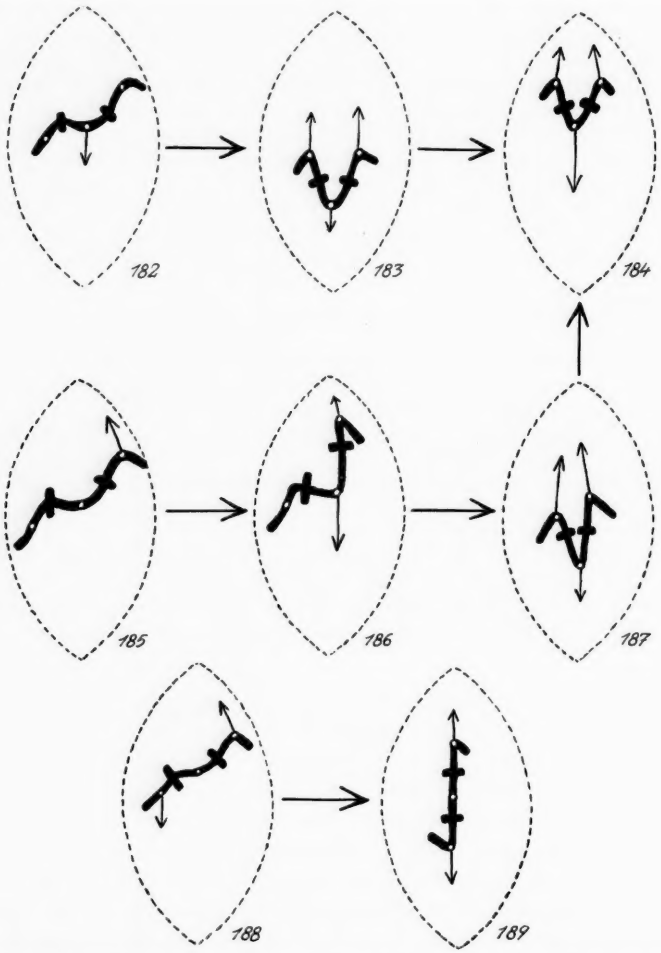
This reasoning was applied to the first division of meiosis. Probably the auto-orientation at the mitotic division takes place more easily and more rapidly than the co-orientation just discussed. But it may still be good to have a margin of safety before the onset of anaphase, and so the above reasoning may be well applicable to the events of ordinary mitosis as well.

There is one more function conceivable of the metaphase stage. This function is connected with the question why the partner chromosomes of the bivalents do not start to behave as univalents again when they have separated from one another at the onset of anaphase. Naturally, the strict poleward orientation of the kinetochores produced by the co-orientation makes it very likely that the chromosomes go straight on to the poles after they have separated from one another. If the kinetochore constitution of the paired chromosomes were identical with that of the univalents, there might, however, still be some possibility that they might start irregular movements to and fro on the spindle again. Now, however, the poleward pull working on the paired chromosomes is known often to produce a characteristic deformation

of the kinetochore region of the chromosomes. As mentioned earlier, the kinetic granules are elevated on attenuated threads over the chromosome surface. The shape of the attenuated chromosome bridges found in inversion heterozygotes indicates that the chromosome body has not only elastic but also some degree of *plastic* properties. It is probable that some of the kinetochore deformation in the paired chromosomes is also of the plastic kind and that it will consequently remain after the onset of anaphase. During metaphase all the four kinetic granules of a paired chromosome have been pulled in the same direction. The plastic deformation of the kinetochores may then result in such an arrangement of the kinetic granules that all of them are turned more definitely in one single direction. The kinetochores may in this way lose their ability to orient their kinetic granules alternatively towards a single pole or towards both poles at the same time. It was this ability of alternation which conditioned the irregular distribution of the univalents, and with its loss the separating partner chromosomes have no possibility left to deviate from a regular anaphase movement. If there is a plastic deformation of the kinetochore of this kind, then the functional purpose of the metaphase may be to give the α-factor sufficient time to produce this useful deformation.

In the univalents these deforming forces will be expected to give quite a different effect, as already mentioned above. Here, the kinetic granules are often oriented in such a manner during metaphase that they are pulled in both of the two opposite poleward directions. Then the deformation will result in an increased separation of the kinetic granules, leading to an increased ability of them to orient towards opposite poles (Figs. 132—133). This leads to the well-known movement of the univalents towards the equator in the late stages of the first division, and if the forces are sufficiently strong or the association of the daughter kinetochores sufficiently weak, then it also leads to the well-known division of the lagging univalents (*cf.* above).

The co-orientation in multivalents now remains to be discussed. The way in which the chromosomes of multivalents orient depends much upon the manner in which they are linked to one another by chiasmata. Thus, there are trivalents of different types: *e. g.*, the chain type, the Y type, the frying pan type, and the triple arc arrangement. Trivalents of the chain type usually take up the V arrangement, but as an alternative they may sometimes assume a rod arrangement at metaphase. The frying pan type is usually arranged with the two chromosomes of the ring oriented towards opposite poles and the third chromosome oriented towards one of the two poles, but such trivalents may occasionally be arranged with both the partners of the ring directed towards the same pole and with the third chromosome oriented towards the opposite pole. These various types of arrangement of the chromosomes are generally well-known, and they need not be further elucidated here by the presentation of a literature review on the subject. I have studied these effects myself in triploid *Anthoxanthum aristatum* and in triploid rye (ÖSTERGREN, unpublished).



Figs. 182—189. Diagrams showing the mechanism of co-orientation in chain trivalents. The kinetochore regions are denoted by white dots on the chromosomes. The arrows at the kinetochores indicate forces, and those between the spindles show the course of the events (the seriation of the figures). — Figs. 182—184. A series leading to a V-arrangement of the chain. In Fig. 182 the central kinetochore has happened to start active movement towards the lower pole. As a consequence (Fig. 183) it orients its two partner kinetochores towards the opposite pole (by pulling at them, *cf.* Figs. 151—154). The final result is seen in Fig. 184. — Figs. 185—187 and 184. Another series giving the same result. — Figs. 188—189. A series leading to a rod arrangement. The right and the left kinetochore in Fig. 188 have happened

The preference for the V arrangement shown by the chain trivalents is only a special case of the general tendency of chromosomes that are materially associated to orient towards opposite spindle poles, a tendency well-known from the preference of quadrivalent and multivalent rings to assume a regular zig-zag arrangement.

The V arrangement of the chain trivalents is explained by the orientation-by-pulling hypothesis as due to a dancing around of their chromosomes during prometaphase which gives the result that chromosomes paired with one another come to exert a pull on one another and as a consequence of that will become oriented towards opposite poles (Figs. 182—187). The rod arrangement which is alternative to the V one is due to the circumstance that both the two terminal chromosomes of the chain have happened to start a simultaneous movement towards the two opposite spindle poles (Figs. 188—189). Then the kinetochore of the middle chromosome will be pulled simultaneously towards both poles and it may fail to orient successfully towards either one of them. Probably it will remain in a similar »unoriented» condition as often seems to characterize univalent chromosomes. This interpretation may also be applicable to certain rod-shaped T-bivalents, having an active T-end in one of the two partner chromosomes (ÖSTERGREN and PRAKKEN, 1946, p. 485 and Fig. 14, p. 482). Nor do the other types of trivalents mentioned above present any special difficulties when we interpret the origin of their orientation in terms of the present theory.

The study by HUGHES-SCHRADER (1943 b) on the behaviour of the sex chromosome trivalent in mantids during prometaphase has given results in good agreement with the present hypothesis. When the sex trivalent comes in contact with the spindle its chromosomes are paired in an end-to-end alignment (X_1-Y-X_2). The sex chromosomes are at first attracted at random towards the two opposite spindle poles and they are later re-oriented to give a regular V arrangement with both the X's directed to one pole and the Y oriented towards the opposite one. I cite from HUGHES-SCHRADER (*l. c.*, p. 273): »Perhaps the most baffling feature of the metaphase orientation lies in the movements of the sex trivalent. We have seen that it assumes, at random, a variety of orientations during the stretching process. If the two Xs have moved to one pole and the Y to the other, the movement to the equator proceeds as in the bivalents simply by contraction of the chromosomes and a shifting of the whole configuration, with its orientation unaltered, into position in the metaphase plate. But if any other orientation be assumed during the stretch — if one X goes to each pole with the Y stretched between them, or if one X and Y pass to one pole

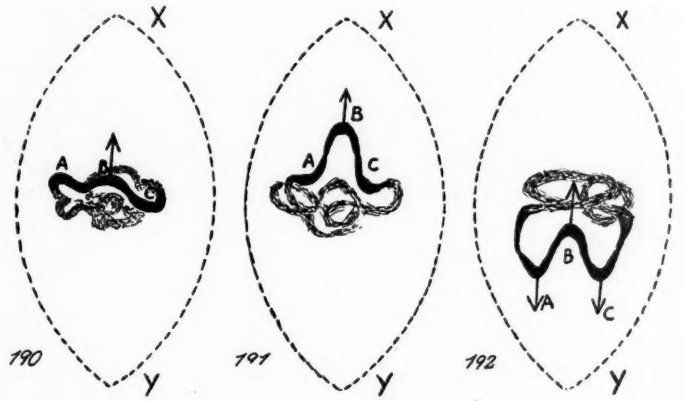
to start simultaneous movements towards opposite poles. Consequently the central kinetochore will be pulled simultaneously towards both poles and it will be unable to orient towards any of them.

while the other X goes to the opposite pole — a re-orientation ensues. One or more kinetochores shift their position and move through the spindle so as to bring the two Xs opposite to the Y on either side of the equator. Thus one kinetochore actually changes its orientation from one pole to the other and moves from a position close to one center to the opposite side of the equator — a maneuvre difficult indeed to visualize in terms of the mechanics of mitosis . . . What happens to the half spindle components during re-orientation would be of great interest, but I have not been able to follow it. Half spindle fibers are occasionally clearly visible during the stretch and are again well marked at metaphase; it is possible that they are lost and reform anew during the re-orientation.»

It is of special interest to notice her remark concerning the »half spindle components», as it indicates that the kinetochores are capable of turning around into a new orientation in spite of being attached to chromosomal spindle fibres. This is in good agreement with the results reported above.

It is often occurring in trivalents that two of the three kinetochores are linked at a much shorter distance to one another than to the third one, because of differences in the length of the chromosome arms or because of the localization of the chiasmata. In such cases the two kinetochores that are closely connected to one another show a strong preference to being oriented towards opposite poles; the third kinetochore is then usually oriented towards the same pole as one of the two others. This arrangement is, of course, quite as expected from the orientation-by-pulling theory. Because of the shortness of the connecting chromosome region the two kinetochores close to one another will interfere much more vigourously with their mutual movements and they will consequently rapidly orient towards opposite poles. It can even be found in such cases that the third chromosome sometimes fails to assume any normally poleward orientation; it remains in a similar »unoriented» condition as is usually characteristic of univalents. In such cases the two kinetochores close to one another have co-oriented and become stabilized in the metaphase equilibrium without exerting a sufficient pull on the more remote partner of the trivalent. I have observed such a failure of co-orientation of chromosomes engaged in trivalents in *Tulipa Gesneriana* and *Anthoxanthum aristatum*. Such an effect has also been reported by others, *e. g.* DARLINGTON (1936 a) working on *Fritillaria* and UPCOTT (1937 b) studying *Tulipa*.

The »disjunctional» zig-zag arrangement at metaphase characteristic of the complex rings of *Oenothera* and *Rhoeo* is explained by the present hypothesis as due to a dancing around of the chromosomes at prometaphase, during which process the chromosomes by mutually pulling at one another come to be oriented in a direction opposite to their neighbours. Probably one of the chromosomes in a ring has origin-



Figs. 190—192. An interpretation of the mechanism leading to a regular zig-zag arrangement in complex rings such as found in *Oenothera* and *Rhoeo*. Attention is fixed on those three of the chromosomes where the regular arrangement (by a random coincidence) happens to start. The other chromosomes are only faintly indicated in the figure. — Fig. 190. In one of the chromosomes (*B*) of the ring the poleward orientation has happened to be a little more perfect than in the others, just as expected from random orientation. This chromosome starts to move towards the pole *X*. — Fig. 191. *B* is pulling at its two neighbours *A* and *C* and thus orients them towards *Y* (by the mechanism suggested in Figs. 151—154). — Fig. 192. As *A* and *C* are two and *B* only one, the group *A-B-C* is pulled over towards the pole *Y*. *A* and *C* then exert a pull on the next chromosomes in the ring, and these are oriented towards *X*. In this way the orientation gradually proceeds all around the ring, through a pendulation to and fro in the two poleward directions.

ally happened to have a somewhat more distinct orientation towards a pole than the others, as would be expected to occur in most cases on the basis of mere randomness. Then the co-orientation will start at this chromosome and proceed around the ring until all its chromosomes have been oriented (Figs. 190—192). If the orientation happens to start simultaneously at two different points, a non-disjunctional arrangement may arise in the ring, as is sometimes found. It is clear that this mechanism of co-orientation in rings will work well only when the kinetochores have a median localization in the chromosomes and when the chiasmata are terminalized. If the chromosome arms are of unequal length, it might easily happen, as in the trivalents mentioned above, that a pair of kinetochores closley associated to one another co-orient and become stabilized in the metaphase position without exerting a noticeable influence on their more remote neighbours. Interstitial chiasmata have the result that the distances between the kinetochores

become unequal owing to the variable chiasma localization, and this arrangement may consequently result in the same kind of disturbance. It is noticeable that *Oenothera* and *Rhoeo* have chromosomes with median kinetochores and terminalized chiasmata. The differences found in the frequency of the »disjunctional» arrangement between interchange heterozygotes of different organisms may largely be due to factors of this kind. Similar considerations are presented by DARLINGTON (1937 a, pp. 151—153), but as they are based on his hypothesis of electrostatic kinetochore repulsions they cannot be maintained in their details.

The actively mobile chromosome ends (T-ends) found in certain strains of inbred rye (KATTERMANN, 1939; PRAKKEN and MÜNTZING, 1942; ÖSTERGREN and PRAKKEN, 1946) obviously behave as kinetochores on the spindle. Consequently it seems adequate to state that the T-bivalents, although bivalents from a genetical standpoint, are multivalents from a mechanical point of view. It is clear that it may be quite appropriate to consider certain features of the behaviour of the T-chromosomes as cases of co-orientation of kinetochores and T-ends, as was done by ÖSTERGREN and PRAKKEN (1946).

The mechanism suggested here to explain the co-orientation does not assume any special kind of forces by which the first meiotic division would differ from ordinary mitotic divisions. The spindle and the kinetochores function in quite the same way during both types of division, and the difference in chromosome behaviour is merely due to a slight difference in the organization of the kinetochore region of the chromosomes. Consequently it is in no way surprising to find that a co-orientation of quite the same kind as the meiotic one can also take place in ordinary mitotic divisions, if only the necessary structural organization of the chromosomes is present. This effect has been found by MELANDER (1950) in the parasitic crustacean *Ulophysema öresundense*. The phenomenon was explained by MELANDER in accordance with the hypothesis of the present paper (which he cited from my, at that time unpublished, manuscript).

VIII. NOTES ON MATERIAL AND METHODS.

The material used in the present study was selected on the simple rule of availability and suitability. Plants that were cultivated for other reasons in Svalöf by the Institute of Genetics of Lund or in the Botanical Garden of the University of Lund were used. Some material cultivated at the Institute for Cell Research of Karolinska Institutet, primarily for other studies, was also used in the present work, and some commonly available varieties of flower bulbs were bought for this study.

In the case of *Tradescantia*, *Trillium* and *Lilium candidum* only temporary aceto-carmine slides were used, but the other materials were fixed in chrome-acetic formalin (the modification by MÜNTZING, 1933), embedded in paraffin and cut into sections. The root tips were fixed directly in this fixative, but the flower buds were prefixed in acetic alcohol (1 : 3); in the case of *Lilium* and *Tulipa*, however, no prefixation was applied, but the anthers were cut into pieces, which were immediately dropped into the chrome-acetic-formalin fixative. The slides were stained according to the ordinary crystal violet method, according to Feulgen (in a few cases), or with acetocarmine (*cf.* EHRENBERG and ÖSTERGREN, 1942), or, finally, a double stain of acetocarmine and crystal violet was used in some cases (*cf.* ÖSTERGREN and PRAKKEN, 1946). The last two methods were used to make the spindle more easily visible.

In the present connection the special cytology of the individual materials used is not of much interest. It is not necessary to fill a lengthy space here with reviews of various authors, who have counted the chromosome numbers of these species, *etc.* Many of the materials are quite commonly used in various cytological work, and their general characteristics can be presumed to be widely known.

The following materials were used; when no special variety is stated, ordinary population material is meant: *Scorzonera hispanica*, *Paeonia officinalis*, *P. anomala* × *tenuifolia* ($2n = 10$; a hybrid cultivated in the Botanical Garden of Lund; its number of bivalents varies from 0—5, with 3 as the most common frequency), *Butomus umbellatus* (triploid or *ca.* triploid material growing near Svalöf), *Anthoxanthum aristatum* (diploid and triploid; *cf.* ÖSTERGREN, 1947 b), *Secale cereale* (normal plants of »Stålråg»; partially asynaptic plants of »Stålråg» studied in the slides of PRAKKEN, 1943; a partially asynaptic plant of »Östgöta Gråråg» containing 2 B chromosomes of the large iso-fragment type; *cf.*, *e. g.*, MÜNTZING and LIMA-DE-FARIA, 1949; this plant was found by the present writer in material received from MÜNTZING; a slide of triploid rye, inbred and having T-chromosomes; the same slide was earlier studied by PRAKKEN and MÜNTZING, 1942, and ÖSTERGREN and PRAKKEN, 1946; a slide of haploid rye earlier used in the work of LEVAN, 1942), *Triticum vulgare* × *turgidum* (an F_2 plant, $2n = 38$), *Triticum vulgare* × *Secale cereale* (fixation made on an F_1 plant cultivated by MÜNTZING for plant breeding purposes), *T. vulgare* × *S. cereale* (amphidiploid, the type »*Triticale* A» of MÜNTZING, 1939), *Tradescantia virginiana*, *T. paludosa*, *Trillium erectum*, *Lilium tigrinum* (ordinary triploid garden material), *L. candidum*, *L. candidum* × *chalcedonicum* (a common garden hybrid known as »*L. testaceum*» — its cytology has been studied by RIBBANDS, 1937), *Tulipa Gesneriana* (the triploid variety »Keizerskroon»), *Allium Cepa* × *fistulosum* (a hybrid produced by LEVAN and studied by him, 1936, 1941; my fixation was made on the plants of LEVAN), *A. Cepa*, and *Hyacinthus orientalis* (ordinary diploid and triploid garden varieties). Testes of the newt *Triturus vulgaris* were also used.

Acknowledgements. — For materials used in the present study I am indebted to many persons, especially Dr. A. LEVAN and Professors A. MÜNTZING and R. PRAKKEN, a fact which is gratefully acknowledged.

IX. CONCLUSIONS AND SUMMARY.

The problem of the co-orientation is an essential part of the more extensive problem of why the chromosomes behave during the first

division of meiosis in quite a different way than they do in ordinary mitotic divisions. The first meiotic division differs from ordinary mitosis by the fact that the chromosomes are linked to one another by chiasmata. But this association of the chromosomes to one another is not in itself a sufficient condition to produce the characteristic difference in chromosome behaviour. It is a well-known fact (*cf.*, *e. g.*, DARLINGTON, 1937 a) that dicentric chromosomes behave as ordinary mitotic ones during the metaphase of mitosis. And even the *univalents* of the first meiotic division behave in a way which is much different from that of mitotic chromosomes. The essential difference between these two types of division, the difference responsible for the co-orientation, must be a difference in the structure of the organ of movement itself.

A structural difference of this kind was actually demonstrated in the present paper by observations on the kinetochore region. During ordinary mitosis the kinetic granules (»spindle spherules» or what we may choose to call them) are arranged on two opposite sides of the chromosome, but during the first meiotic division the whole kinetochore is arranged on one side of the chromosome body. This arrangement may, in many cases at least, be produced simply by a different manner of coiling of the chromonemata, in ordinary mitosis the kinetochore region shows negative heteropycnosis, but during the first meiotic division this region is isopycnotic (*i. e.* coiled in the same manner as the rest of the chromosome), or it is at least much less constricted than in ordinary mitosis.

In this way the chromosome body is screening off the kinetochore during the first meiotic division, so that it can interact well with the spindle in one direction only.[1] As a consequence, there will be difficulties for the kinetochore to orient simultaneously towards both poles; so, besides such orientations towards both poles, the univalents will often orient more or less definitely towards a single pole only. This fact in combination with the poleward attraction of the kinetochore

[1] It is well possible and perhaps even probable that there may exist other mechanisms which *also* contribute to produce this unilateral direction of the interaction of the meiotic kinetochore with the spindle. Thus, it is quite likely that the kinetic granules themselves have developed a »front side» and a »back side», and also that these sides may show characteristic differences in their orientation between meiosis and ordinary mitosis. It is clear that it may be very difficult to get definite information on such subtle details. The observations made by MELANDER on mitosis in *Ulophysema* indicate such a differentiation of the sides of the kinetic granules, and this differentiation may also be suggested by the pin-like drawn-out kinetochores of plant meiosis.

(the α-factor of ÖSTERGREN, 1949 c) gives rise to the random distribution of the univalents over the spindle during first metaphase. Changes in kinetochore orientation probably cause irregular movements of the univalents to and fro on the spindle. The quadruple structure of the kinetic apparatus of the metaphase chromosome has probably the functional purpose of supplying an apparatus which in combination with the α-factor can produce such irregular movements of the meiotic chromosomes.

The bivalents differ from the univalents by having their kinetochores oriented in quite a definite way during full metaphase. The kinetochores of the two partners are regularly oriented towards the two opposite poles. This orientation of the kinetochores towards opposite poles actually constitutes the essential feature of the co-orientation. The co-orientation concept was given a new definition in accordance with this opinion (above, pp. 88—89). The origin of this difference between univalents and bivalents is the essential problem of the co-orientation. The two partners of the bivalents are strongly attracted towards the two opposite poles towards which they are oriented (by the forces of the α-factor). The strong forces striving to separate the partners at metaphase are a simple consequence of this fact. There is no reason to assume a repulsion between the kinetochores.

The co-orientation is not due to a repulsion between the kinetochores, because no interaction of this kind is found between the kinetochores of univalents on the spindle. The interaction which leads to the co-orientation appears to work through the material connection between the paired chromosomes, as it leaves the univalents uninfluenced.

When paired chromosomes first meet the spindle, they behave just like univalents, *i. e.* they are moved around at random by active kinetochore movements directed towards the one pole or the other, or they may be found resting in a more or less »indifferent» manner on the spindle, probably in that case having their kinetic granules oriented simultaneously towards both poles. Thus, both the kinetochores of a bivalent can sometimes be seen to move actively towards the same spindle pole (during prometaphase). In slides of fixed and stained materials these movements of the univalents and the bivalents can be recognized from the deformations which they cause of the chromosomes. The univalents are just like the paired chromosomes attached to the fibre structure of the spindle.

During these random movements the kinetochores of the paired chromosomes, sooner or later, happen to exert a pull on their partners.

This pull, in connection with the one-sided arrangement of the kinetochore on the chromosome body, results in an orientation of the partner kinetochores towards opposite spindle poles, *i. e.* it produces the co-orientation. This mechanism can explain the co-orientation in bivalents and multivalents, and it gives a promising solution even of the regular zig-zag arrangement found in complex rings such as those of *Oenothera*.

This interpretation has often been called a hypothesis earlier in this paper, but, if all the evidence is considered, it appears that it might well be appropriate to call it a *theory*, the theory of orientation by pulling, which explains the co-orientation in bivalents and multivalents.

Of course, the fact that a kinetochore is seen to be oriented towards the pole towards which it appears to be pulled is not in itself a proof that the *direction* of the pull is a consequence of the *orientation*. It might also be possible that, instead, the kinetochore orientation was resulting from a pull, which in its origin was independent of the original orientation of the kinetochore. If so, we would, however, be left without any idea of what caused the *direction* of the pull, and furthermore all the other phenomena discussed in the present paper would remain unexplained. Consequently, it will be impossible to suggest this idea as an alternative to be considered when we are looking for various interpretations that might explain the co-orientation. Also, there is no evidence of any kind suggesting this reversed direction of causality.

It would be interesting to know how kinetochores furnished with fibre structure towards one pole can turn around and, instead, establish such fibres in the opposite direction. It does not appear very probable that the cell is »wise» enough to dissolve the fibres just when the kinetochore is due to turn around. It would be more probable to suppose rhythmical changes of structure in the fibres, between a gelated state when they are pulling and a liquid one when they can permit the kinetochore to turn. Or, perhaps, the chromosomal spindle fibres are of such a kind that they can exert a pull all the time and still be gradually and continuously reconstructed during the progression of the rotatory movement. They could be suspected to have such properties if they were of some kind of liquid crystalline constitution as suggested in the speculations of ÖSTERGREN (1949 b). However interesting and important this question may be, it need not be considered in more detail in the present connection.

The co-orientation is just as important a detail of the mitotic events as the anaphase movement, and it must be duly regarded by any hypothesis striving at a mechanical analysis of the chromosome movements.

It is remarkable how little this process has actually been considered by the authors working on the problem of the chromosome movements; it would seem that the great majority of them do not even know that there exists a problem of co-orientation. You can neither find the word »co-orientation» nor »orientation» in the index of the monograph of SCHRADER (1944), and the only sentence in his text that looks like a reference to the co-orientation effect is given when he reviews DARLINGTON's hypothesis. It is a prominent contribution by DARLINGTON (1936 a, 1937 a) that he has realized the importance of this effect and tried to give it an explanation, even though his hypothesis can scarcely be regarded as very successful.

A brief review of certain essential points of my above theory was made by MELANDER (1950), who with my permission cited these ideas from the unpublished manuscript of the present paper. The »orientation by pulling» idea including its application to the *Oenothera* rings was actually presented in public by the present writer as early as in 1948, *viz.* in the *discussion* following upon the paper read by me before the Eighth International Congress of Genetics. (All discussions from this congress were, however, excluded from publication in the proceedings.)

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THE GENETIC EFFECT OF NEGATIVE SELECTION IN MAN

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INTRODUCTION.

THE problem of the biological effect of elimination of character-carriers from propagation in man is but a part of the general problem of selection. What is true of man is, of course, principally applicable to all cross-fertilizing organisms. Nevertheless, as far as man is concerned, the problem has several special aspects. There is no need to enter into details, but two points may be observed. On the one hand we have, partly owing to the slow reproduction rate of man, partly from other causes, a rather weak interest for selectional effects noticeable only in the very remote future, after hundreds or thousands of generations. (If there were any inclination for such speculations, the events of the last years, and the uncertain future of mankind, ought to have effaced them.) On the other hand, the problem to a certain extent coincides with the problem of the biological effect of sterilization of hereditary defective individuals. This problem was the starting point of the present investigation, and I had, as a matter of fact, intended merely to write a short review of this question (to provide misunderstanding, I must explain that I have never shared the belief, once widely spread, that an important improvement in the »race» is possible by means of sterilization). Gradually, however, it proved desirable to elucidate the subject more thoroughly and from a purely theoretical point of view. The following calculations are often made on the basis of extreme assumptions never realizable in man (or in any organism). The problems then, to a certain extent, are analysed merely for the sake of their theoretical interest. The results, however, tend to enlighten what really occurs or may occur in human populations. The discussion of the theoretically or (in one single case) to a certain extent even practically possible effect of sterilization on the frequency of the most common and important hereditary defects and diseases (feeble-mindedness, mental diseases, *etc.*) will be given in a future paper.

The nature of the theoretical problems discussed in the following lies in the very facts of general and human genetics. To be able to judge the theoretical effects of elimination of character-carriers from reproduction, we have to analyse this effect under various assumptions as to mode of inheritance, frequency and distribution of the postulated genes, *etc.* I think I have touched on the most important of these merely formal problems as far as has been possible without applying subtle mathematical methods and theory. I lay no claim to having paid sufficient regard to the literature in which such a theory prevails but am simply arguing, to the best of my ability, on the basis of a certain scientific common sense.

I am much indebted to my friend Professor G. DAHLBERG, Head of the Swedish State Institute for Race-Biology, for valuable discussions and for the copies of the diagrams made in his institute. I also express my gratitude to my friend Professor I. SPERBER for an important suggestion concerning a problem of equilibrium.

MONOMERIC INHERITANCE.

MONOMERIC AUTOSOMAL RECESSIVITY. TOTAL SELECTION.

The theory of monomeric autosomal recessivity, where it concerns cases in which the genetic balance is supposed to be undisturbed, has been thoroughly elaborated. What is said below has the purpose mainly to serve as a basis of the following discussions and to facilitate an understanding of the concept for biologists not entirely at home in this field of genetics.

When a character is due to a single autosomal recessive gene, there is, as is well known, in a strictly out-bred population a fixed relation in the frequency between the three types of individuals, the dominant homozygotes *DD* (or *AA*), the heterozygotes *DR* (*Aa*), which in cases of complete dominance cannot be distinguished phenotypically from the dominant homozygotes (are normal if the character in question is a defect), and the (defective) recessive homozygotes *RR* (*aa*). Hence, from the frequency of the latter the frequency of the heterozygous carriers of the gene can be concluded. As a point of departure of the following account a few examples are given in Table 1.

The data illustrated in this table are familiar to anyone acquainted

TABLE 1. *Monomeric autosomal recessivity. Relative frequencies in panmixia (amphimixis) of genes and genotypes. All numbers except the figures in the lowest line in per cent.*

R	50	10	3,16	1,41	1,0	0,707	0,316	0,1
RR	25	1	0,10	0,02	0,01	0,005	0,001	0,0001 [1]
DR	50	18	6,12	2,79	1,98	1,404	0,63	0,20 [2]
DD	25	81	93,78	97,19	98,01	98,591	99,37	99,80
DR : *RR*	2×	18×	61×	140×	200×	281×	630×	2000×

[1] 1 in 1 million. [2] 2000 in 1 million.

with the elementary facts of genetics. For the sake of shortness and in regard to what is of interest in man, the recessive character may be assumed to be a defect. The lower the frequency of the recessive gene and consequently of the defective homozygotes, the greater is the preponderance of the normal carriers of the gene. With a frequency for *RR* of 1 per cent, *DR* are but 18 times as much as *RR*, with a frequency of 0,01 per cent they are almost 200 times this, and with a frequency of 0,0001 they are 2000 times as much as *RR*.

The columns 4—7 in the table are interesting since many hereditary defects in man have a frequency of these orders of magnitude. Two examples may suffice. The frequency of hereditary deaf-mutism is not known but can be roughly estimated to 0,02 per cent. The frequency of so-called retinitis pigmentosa (dystrophia retinae pigmentosa), the well-known form of hereditary blindness, is also unknown but lies undoubtedly considerably below 0,01 per cent, presumably in the neighbourhood of 0,005 per cent or still lower. (The most common, autosomal and recessive type is meant and its genetic unity, which is not proved, is postulated. Calculations considering allowance for age of sickening and age distribution of the population would have too uncertain a foundation and can be omitted, as the genetic frequency can hardly be markedly higher than 0,005 per cent.)

In the case of such rare defects elimination of the defective individuals from propagation has obviously a very insignificant effect. Where the heterozygotes are a hundred or many hundred times as numerous as the defective, removal of the latter implies an exceedingly small or practically a lack of elimination of the defective genes.

On account of the fixed relation between the frequency of homozygotes and that of heterozygotes it is possible to calculate the effect of

TABLE 2. *Monomeric autosomal recessivity and panmixia. Elimination of the recessive homozygotes. Frequencies in per cent.*

Initial frequency of the recessive homozygotes Gen. 0	After 1 generation Gen. I	After 2 generations Gen. II	After 5 generations Gen. V	After 10 generations Gen. X	After 20 generations Gen. XX
2	1,54	1,215	0,686	0,343	0,136
1	0,826	0,694	0,444	0,250	0,111
0,25	0,227	0,206	0,166	0,111	0,062
0,1	0,094	0,088	0,075	0,058	0,038
0,02	0,0194	0,0189	0,017	0,015	0,012
0,01	0,0098	0,0096	0,009	0,008	0,007
0,005	0,005	0,005	0,0047	0,0044	0,0038
0,001	0,001	0,001	0,00097	0,00094	0,00088

an elimination, repeated in several generations, of all individuals suffering from a monomeric recessive defect or showing another recessive character. HALDANE (1924 and later papers) has analysed this and other problems of selection. His account has a subtle and somewhat abstruse mathematical form. A clear account with easily available formulae was given, independently of HALDANE, whose paper of 1924 was at first very little known, by two Swedish scientists, W. HULTKRANTZ and G. DAHLBERG (1927); the mathematical elaboration of the problem seems to be due to the latter, who has later proceeded further in this direction (DAHLBERG, 1926 and later papers. *Cf.* also HOGBEN, 1946). — It is mostly overlooked that already in 1916 JENNINGS had solved this problem, which was not quite as simple then as it seems now, and given a somewhat more complicated but in reality identical formula.

In Table 2 some examples are given of calculations by means of this method, random mating being postulated. All figures indicate the frequency in per cent. As all hereditary diseases and defects are more or less rare, examples are given only of frequencies of up to 2 per cent. »Gen. I» signifies the frequency in the first generation after that in which the elimination is performed, *i. e.* the children generation, »Gen. II» the frequency in the second (grand children) generation, *etc.*

The table and the diagram in Fig. 1 elucidate the law valid for monomeric recessivity: the lower the frequency of a recessive defect (and gene), the less is the reduction caused by the elimination of the character-carriers (the defective). The application of this rule to the two examples given above is rather suggestive. The number of retinitis sufferers would, if the frequency is 0,005 per cent as assumed before —

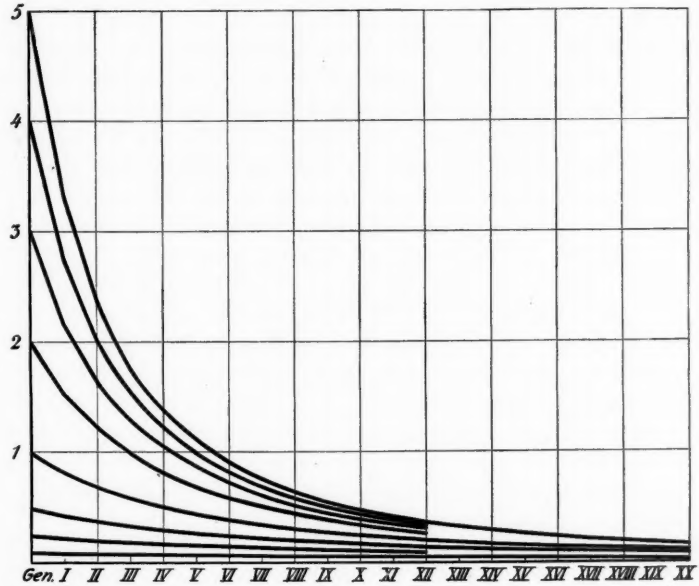

Fig. 1. Monomeric autosomal recessivity and panmixia. Total elimination of the recessive homozygotes. Frequencies in per cent. For four frequencies the curves are drawn only to Gen. XII.

and it cannot be so far from this figure as to contribute an untoward influence on the result — still after elimination, for instance by means of sterilization, of all affected individuals for ten generations (more than 300 years) have decreased only from about 50 to 44 per million. It can be added that the frequency would not be halved in 50 generations (more than 1600 years). Calculations referring to such long periods have, of course, a purely theoretical interest. The frequency of hereditary deaf-mutism would decrease somewhat more, yet only from about 200 in 100.000 individuals to 175 in five and to 150 in ten generations. The effect diminishes with each generation. Finally, a limit is attained where the gene occurs mainly in personally normal heterozygotes but is so rare that the chance of two such persons mating is in random mating very small. The defect will appear only on rare occasions, when by chance two heterozygotes meet and have children. When the frequency of a gene has reached this limit (the lowest heterozygote limit; DAHLBERG), elimination of the sparse defective has no effect whatever on the frequency.

It should also be noticed that if a recessive character has a relatively high frequency, for instance of 1 per cent, elimination of the recessive homozygotes from reproduction has an effect which is strictly limited but by no means insignificant. The frequency will in two generations be reduced to 0,7 per cent. There are, however, no monomeric recessive defects of this frequency in man. Nevertheless, this part of the table has a positive interest as regards man. It illustrates, as will be seen later on, roughly or to a certain extent the result of elimination of character-carriers in many cases of di- and polymerism.

Fig. 1 gives a graphic illustration of the effect of elimination of recessive homozygotes in the first 20 generations for some frequencies between 5 and 0,1 per cent. I constructed this diagram long ago and it may still have some interest, in helping to visualize the significance of the facts discussed above.

What is said above holds good for a population mating at random; thus a condition is presumed which certainly is never wholly realized. The influence, if any, of deviations from panmixia will be discussed further on.

MONOMERIC AUTOSOMAL RECESSIVITY. PARTIAL SELECTION.

The principle for calculating the effect of partial negative selection of recessive character-carriers has been analysed by HALDANE, DAHLBERG and HOGBEN, who give »recursion» formulae, the latter two providing tables. I have employed the formula of HALDANE (and HOGBEN) or in most cases made the calculations directly, since I have the impression that this method is almost easier. [DAHLBERG gives a special formula for »fertility selection», *i. e.* the case where the eliminated (sterile) character-carriers are married to other individuals. The different genotypes, however, have the same relative frequency among the fertile individuals, which are in this way excluded from propagation and thus affected by the selection, as in the whole population, and the result is therefore the same as if the character-carriers die or in another way are prevented from sexual alliances.]

The most interesting inferences are those concerning the reduction set in relation to the reduction in total selection. Data for the first generation and an elimination of 50 per cent of the recessive homozygotes are given in Table 3, which embraces frequencies from 99 to 0,01 per cent. They are graphically illustrated in Fig. 2. (In the calculations, here and in the following, more decimal places have been employed than shown in the tables.)

TABLE 3. *Monomeric autosomal recessivity. Effect in the first generation of elimination of 0,5 of the character-carriers. All frequencies in per cent.*

Gen. 0	99	81	64	36	25	16	6,25	4	2	1	0,1	0,01
Gen. I	98,03	69,21	49,62	26,23	18,37	12,10	5,10	3,37	1,76	0,91	0,097	0,0099
Reduct. in per cent of the red. in total elim.	1,3	20,0	32,0	44,5	47,8	49,8	51,16	51,25	51,15	50,9	50,0	50,0

In the highest frequencies approaching 100 per cent *RR* the reduction is insignificant, approaching zero. (In very high frequencies it would, of course, in reality seem more natural to speak about an in-

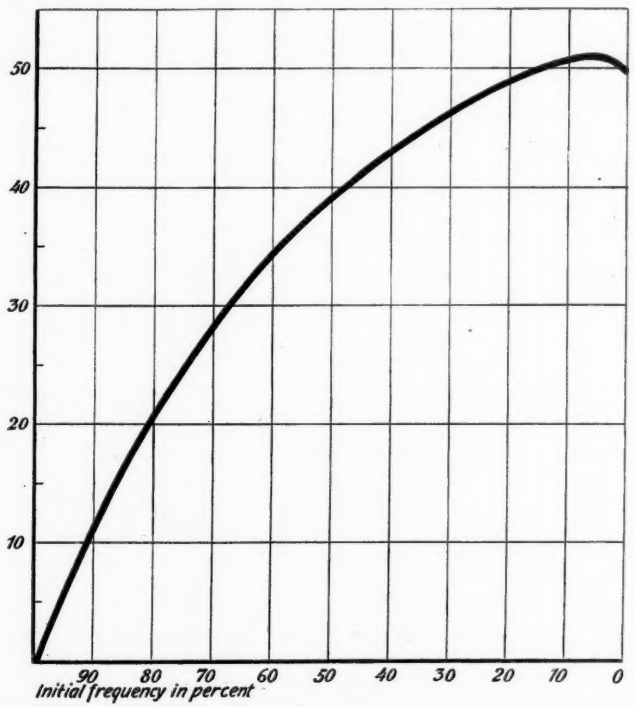

Fig. 2. Monomeric recessivity. Elimination of 0,5 of the recessive homozygotes (character-carriers). Reduction in the first generation in per cent of the reduction in total selection.

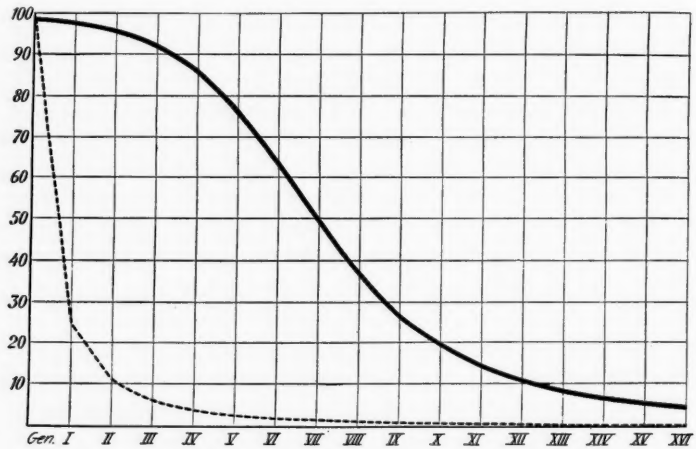

Fig. 3. Monomeric recessivity. *RR* 99 per cent. Elimination of 0,5 of the recessive homozygotes. Below (dashed line), total elimination.

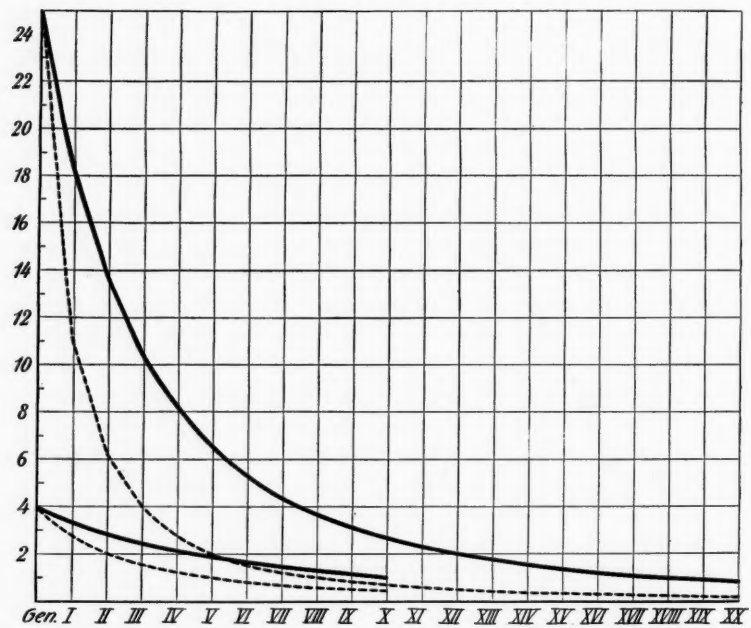

Fig. 4. Monomeric recessivity. *RR* 25 and 4 per cent. Elimination of 0,5 of the recessive homozygotes. Dashed lines, total elimination.

TABLE 4. *Monomeric autosomal recessivity, partial selection. Elimination of 0,5 of the recessive homozygotes as compared with the effect of total selection. 10 generations. All frequencies in per cent. The third line for every initial frequency indicates the reduction in per cent of the reduction in total selection.*

Initial frequency	Elimination	I	II	III	IV	V	VI	VII	VIII	IX	X
99	Total	24,87	11,07	6,23	3,99	2,77	2,04	1,56	1,23	1,0	0,83
	0,5	98,02	96,14	92,64	86,52	76,90	64,00	49,83	37,01	26,98	19,77
	—	1,3	3,24	6,85	13,14	22,87	36,09	50,46	63,34	73,5	80,7
64	Total	19,75	9,47	5,54	3,63	2,56	1,90	1,47	1,17	0,95	0,79
	0,5	49,83	37,0	26,97	19,75	14,71	11,20	8,71	6,92	5,60	4,61
	—	32,0	49,5	63,3	73,3	80,2	85,0	88,4	90,8	92,6	94,0
25	Total	11,11	6,25	4,0	2,78	2,04	1,56	1,23	1,0	0,83	0,69
	0,5	18,37	13,75	10,52	8,23	6,57	5,34	4,41	3,69	3,13	2,68
	—	47,8	60,0	69,0	75,5	80,3	83,9	85,7	88,8	90,5	91,8
4	Total	2,78	2,04	1,56	1,23	1,0	0,83	0,69	0,59	0,51	0,44
	0,5	3,37	2,88	2,48	2,16	1,89	1,67	1,49	1,33	1,20	1,08
	—	51,3	57,2	62,3	66,6	70,2	73,3	75,9	78,2	80,2	82,0
2	Total	1,54	1,21	0,99	0,82	0,69	0,59	0,50	0,44	0,39	0,34
	0,5	1,76	1,56	1,40	1,25	1,13	1,03	0,93	0,85	0,78	0,72
	—	51,1	55,6	59,6	63,1	66,1	68,9	71,3	73,5	75,4	77,2
1	Total	0,826	0,694	0,592	0,510	0,444	0,391	0,345	0,309	0,277	0,250
	0,5	0,912	0,834	0,766	0,706	0,652	0,605	0,562	0,524	0,489	0,458
	—	50,9	54,3	57,3	60,0	62,5	64,9	67,0	68,9	70,7	72,3
0,1	Total	0,0940	0,0885	0,0834	0,0789	0,0746	0,0706	0,0670	0,0637	0,0606	0,0577
	0,5	0,0970	0,0941	0,0913	0,0886	0,0861	0,0836	0,0813	0,0791	0,0770	0,0749
	—	50,0	51,5	52,6	53,6	54,7	55,7	56,7	57,6	58,5	59,4

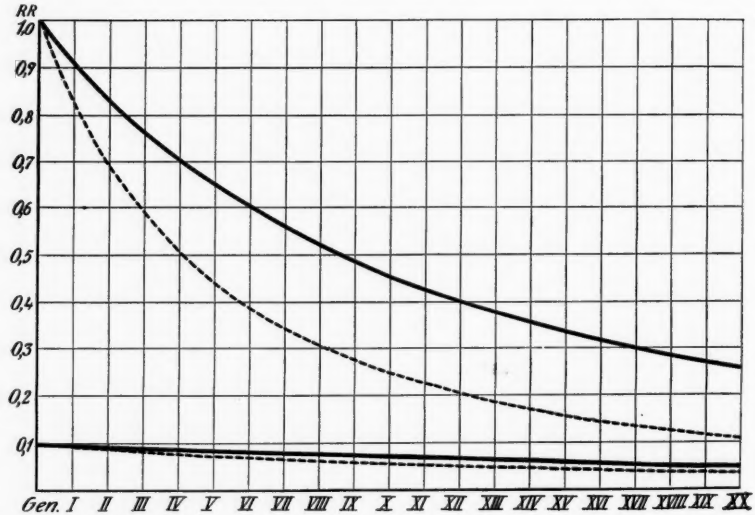

Fig. 5. Monomeric recessivity. *RR* 1 and 0,1 per cent. Elimination of 0,5 of the recessive homozygotes. Dashed lines, total elimination.

creased reproduction of the rare dominant genotypes.) The reduction rises with falling frequency to a gradually larger percentage of the reduction in total selection. A maximum is attained at the initial *RR*-frequency of 4 per cent, where the reduction is 51,25 of that occurring after total elimination. From this frequency downward the reduction decreases a little until the limiting value 50 per cent is attained (Table 3, Fig. 2).

In the following generations the effect of the partial elimination increases gradually in relation to the effect in total selection. Table 4 gives the result for elimination in 10 generations of 50 per cent of the recessive character-carriers in some initial frequencies from 99 to 0,1 per cent. Curves embracing 20, partly 10, generations are for some frequencies given in Figs. 3—5.

Fig. 6 illustrates the relations between partial and total selection in a more perspicuous fashion than Table 4. In all initial frequencies below about 30 per cent (25 to 0,1 per cent in the diagram) the reduction is in the first generation about 50 per cent of that after total elimination (particulars in Fig. 6). It rises in subsequent generations, very slowly for very low frequencies, more rapidly the higher the initial frequency. In frequencies markedly higher than 30 per cent (64 and 99 per cent

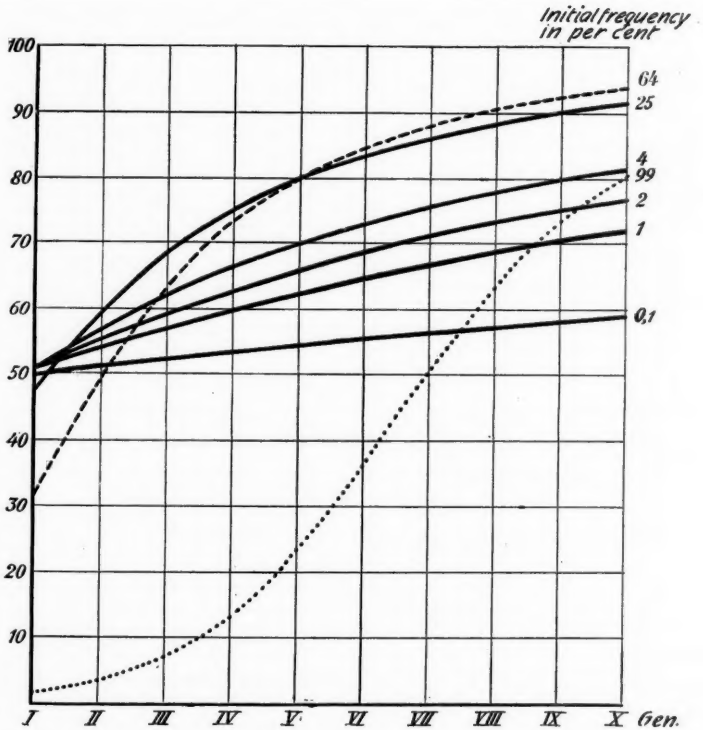

Fig. 6. Monomeric recessivity. Elimination of 0,5 of the recessive homozygotes. Reduction in per cent of the reduction in total selection.

in Fig. 6) the relative reduction is in the first generation much lower and rises very strongly; in the initial frequency of 64 per cent it approaches already in the tenth generation the reduction in total selection.

All this is in principle not difficult to understand. When the frequency of the recessive homozygotes is initially very high, close to 100 per cent, total elimination immediately, in the first generation, reduces the frequency to a very low amount which then, in subsequent generations, can be but slowly still more reduced. After elimination of, for instance, 50 per cent of the character-carriers these have still a great predominance, and further elimination has therefore a strong but gradually diminishing effect. In 0,5 elimination the recessive character-carriers have in the first generations still so great a predominance that their frequency sinks but slowly; after selection in several generations

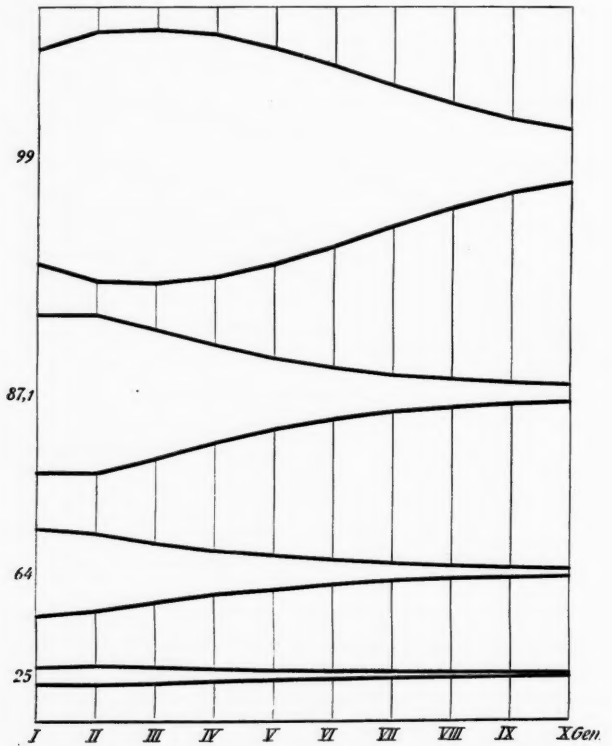

Fig. 7. Monomeric recessivity. Comparison between the reduction in total and 0,5 elimination of the recessive homozygotes, ten generation (distance between the frequency curves). Initial frequencies 99—25 per cent.

their frequency again sinks very slowly but now for a quite opposite reason, namely their great scarcity. This explains the shape of the curve for 99 per cent in Fig. 3; it has the form of an inverse, very extended S. The lower the initial frequency, the greater is the planing out of the difference between the effect of total and partial selection. In extremely low cases for such frequencies, total and partial selection have both from the beginning an almost imperceptible effect and the difference, 50 per cent, is very slowly altered. In all cases, on approaching zero, the effect of total and of partial elimination will sooner or later almost coincide.

The difference between the effect of total and of partial selection thus depends on the initial frequency of the recessive homozygotes. The nature of this dependence

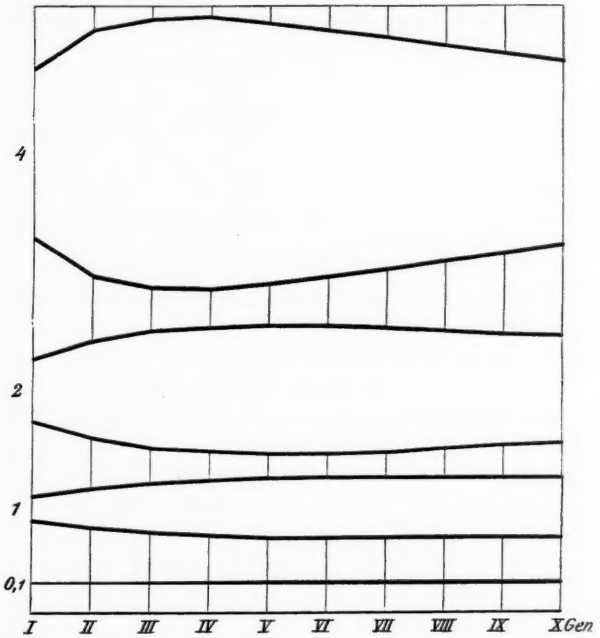

Fig. 8. As Fig. 7 but initial frequencies 4—0,1 per cent. Vertical scale 100 times that in Fig. 7.

bears a consequence which may seem rather surprising: the difference is not equally altered in relation to the change of the frequency but in a different manner for very high, for more moderate and for lower frequencies. The following particulars, illustrated in the diagrams Figs. 7 and 8, may be of some interest. As before, a partial elimination of 50 per cent is assumed.

In very high frequencies (99 per cent in Fig. 7) the curves for partial and total elimination diverge at first (in two generations), then converge (*cf.* also Fig. 3). With falling frequency the divergence diminishes, and the reversal to convergence follows earlier (after one generation). At a gene frequency of 0,9332, which gives a *RR*-frequency of 87,086 per cent, the curves are parallel from the first to the second generation. In lower frequencies they converge from the beginning until a gene frequency of 0,5475, which gives a *RR*-frequency of not quite 30 (29,9756) per cent; this frequency is a turning-point where the curves are again parallel in one generation. From then on they are at first divergent, then convergent (Fig. 8); the reversal becomes later the more the frequency sinks, in a homozygote frequency of 1 per cent in Gen. XI (*cf.* also Fig. 5), in much lower frequencies very late.

To sum op the result for low frequencies which may have special importance for man: elimination of half of the recessive character-carriers gives in initial frequencies of 1 per cent and less a reduction

TABLE 5. *As Table 3 but with 0,1 of the character-carriers eliminated.*

Gen. 0	99	81	64	36	25	16	4	2	1	0,1
Gen. I	98,89	79,42	61,83	34,23	23,73	15,23	3,87	1,95	0,98	0,099
Reduct. in per cent of the red. in total elim.	0,015	2,70	5,13	8,06	9,11	9,77	10,43	10,41	10,34	10,13

which in the first generation is about half of the reduction after total elimination and then is relatively increased, comparatively much at 1 per cent *RR*, less in lower frequencies.

The same general principles hold good for other degrees of partial selection. Table 5, which corresponds to Table 3, gives the data for the first generation at an elimination of one tenth of the recessive homozygotes. The reduction is in very high frequencies extremely small, almost nil, rising with falling frequency to about 10 per cent of that after total elimination. The maximum, 10,43 per cent, is attained at an initial frequency of 4 per cent. From then on, the relative reduction falls a little until the limiting value 10 per cent is obtained.

In Table 6 and Figs. 9—11 the effect of 0,1 elimination is for three initial frequencies compared with the effect of total elimination. The relative reduction (in per cent of the reduction in total elimination) increases gradually in subsequent generations. The increase is great for high frequencies and diminishes with sinking frequency. In the very high initial *RR*-frequency of 99 per cent the relative reduction is in

TABLE 6. *As Table 4 but with 0,1 of the recessive homozygotes eliminated.*

Initial frequency	Elimination	I	II	III	IV	V	VI	VII	VIII	IX	X
99	Total	24,87	11,07	6,23	3,99	2,77	2,04	1,56	1,23	1,0	0,83
	0,1	98,89	98,77	98,63	98,49	98,32	98,14	97,94	97,72	97,47	97,20
	—	0,15	0,26	0,39	0,54	0,70	0,89	1,09	1,31	1,57	1,83
64	Total	19,75	9,47	5,54	3,63	2,56	1,90	1,47	1,17	0,95	0,79
	0,1	61,83	59,64	57,42	55,21	53,00	50,81	48,64	46,50	44,42	42,37
	—	4,90	8,00	11,25	14,56	17,88	21,24	24,56	27,85	31,61	34,21
1	Total	0,826	0,694	0,592	0,510	0,444	0,391	0,346	0,301	0,277	0,250
	0,1	0,982	0,985	0,988	0,991	0,995	0,999	0,994	0,999	0,994	0,990
	—	10,34	11,59	12,84	14,09	15,53	16,57	17,79	19,00	20,19	21,37

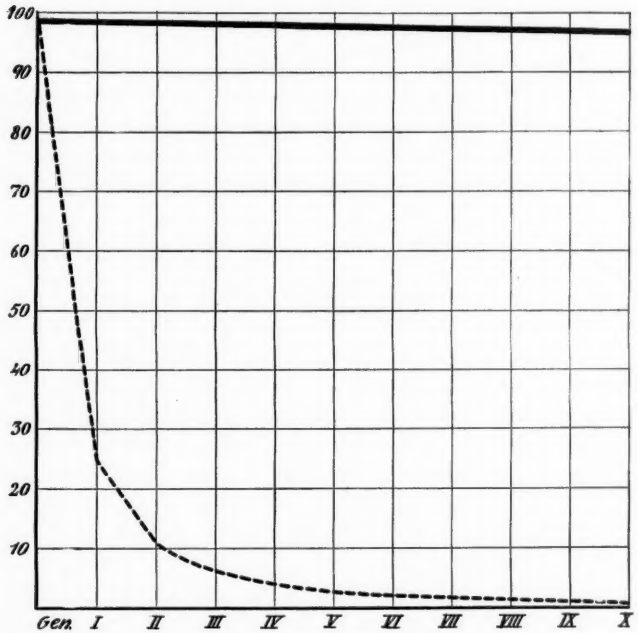

Fig. 9. Monomeric recessivity. *RR* 99 per cent. Elimination of 0,1 of the recessive homozygotes. Dashed line, total elimination.

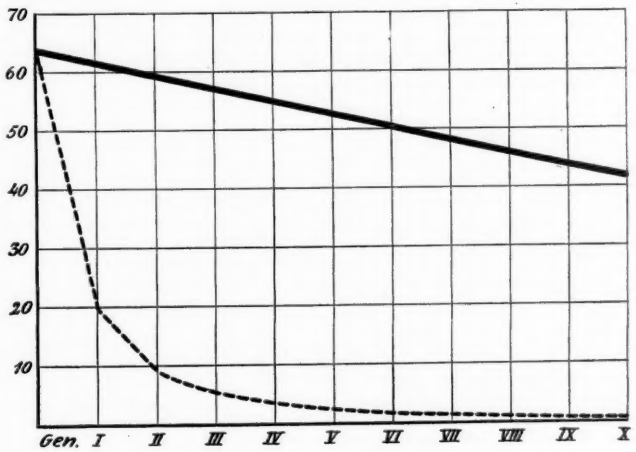

Fig. 10. As Fig. 9. *RR* 64 per cent.

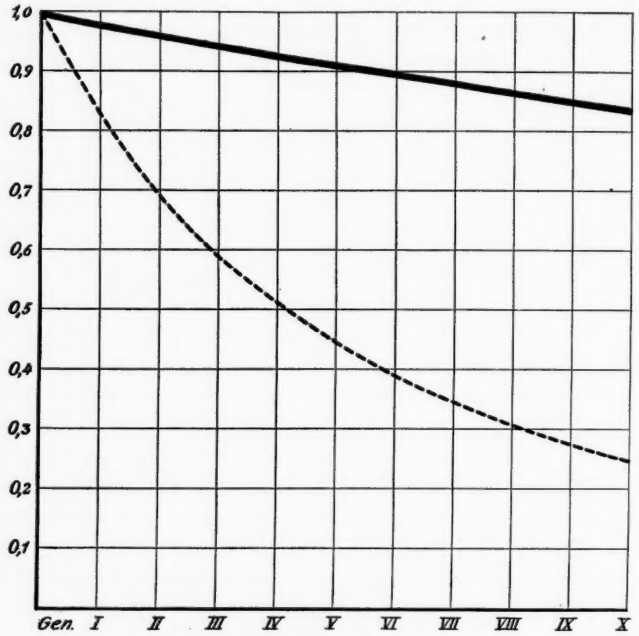

Fig. 11. As Fig. 9. *RR* 1 per cent.

Gen. X more than 12 times the reduction in Gen. I; in a frequency of 1 per cent the corresponding figure is a little more than doubled. Thus, in lower frequencies and for a moderate number of generations, the reduction lies between about 10 and 20 per cent of the reduction in total selection.

In this feeble partial selection the curves for total and partial elimination are always at first divergent and then convergent, never convergent from the beginning as in certain frequencies in stronger elimination (*cf.* Fig. 7; 64 per cent). In very high frequencies (Fig. 9) they are seen to converge extremely slowly from Gen. V. They will meet only after many hundred generations or, more strictly speaking, never meet at all, for when they would almost coincide the curve for total selection would long ago have sunk to the least heterozygous limit, *i. e.* to practically zero (after 100 generations, when in total selection the frequency is hardly 0,01 per cent, it is in 0,1 elimination still very high, something about 70 per cent). In the lower but still very high frequency of 64 per cent (Fig. 10) the convergence begins from Gen. III; the

curves will almost coincide after about 30 generations, when in total selection the frequency is 0,1 per cent. For a frequency of 1 per cent (Fig. 11) convergence will have a late beginning, many generations after the tenth. The curves will almost coincide after presumably about 100 generations, when in total elimination the frequency is brought down under 0,01 per cent.

RECESSIVE SEX-LINKED INHERITANCE. TOTAL SELECTION.

In recessive sex-linked inheritance elimination of the character-carriers, as is well known, has a much stronger effect than in monomeric autosomal recessivity because of the quite different relations between character-carriers and heterozygotes. The latter, females only when the male sex is heterogametic as in man, are in lower frequencies (from $r\ ^1/_3$, character-carriers $^2/_9$) more numerous than the character-carriers but never more than twice as numerous. Elimination therefore must cause a rapid and strong diminution of the frequency. (In the following, as in other cases, sharply limited generations are presumed.)

If a sex-linked defect exists in $1/n$ males, this means, as the males possess one *X*-chromosome only, that the gene, *R*, exists in $1/n$ *X*-chromosomes. The chance for two such chromosomes to meet being $1/n^2$, the homozygote, defective females (*RR*) are $1/n^2$. In other words, if the defective males *RO* are *n* per cent, the defective females *RR* are $n^2/100$

TABLE 7. *Sex-linked recessivity. Distribution of genotypes in different frequencies. Frequencies in per cent.*

♂		♀			Total
DO	*RO*	*DD*	*DR*	*RR*	*RO* + *RR*
1	99	0,1	1,98	98,01	98,505
19	81	2,61	31,78	65,61	73,305
36	64	12,96	46,08	40,96	52,48
50	50	25	50	25	37,5
60	40	36	48	16	28
66,67	33,33	44,44	44,44	11,11	22,22
80	20	64	32	4	12
90	10	81	18	1	5,5
95	5	90,25	9,5	0,25	2,625
96	4	92,16	7,68	0,16	2,08
99	1	98,01	1,98	0,01	0,505
99,9	0,1	99,8001	0,1998	0,0001	0,05005
99,99	0,01	99,980001	0,019998	0,000001	0,0050005

TABLE 8. *Sex-linked recessivity. Elimination in two generations of male character-carriers RO. DR female heterozygotes (conductors). Frequencies in per cent.*

Gen.	*RO*	*DR*	*RO*	*DR*	*RO*	*DR*	*RO*	*DR*
0	1	1,98	0,5	0,995	0,1	0,1998	0,01	0,02
I	1	1	0,5	0,5	0,1	0,1	0,01	0,01
II	0,5	0,5	0,25	0,25	0,05	0,05	0,005	0,005

per cent. From this the frequency of the heterozygote females *DR*, the »conductors», is calculated in the usual way. (*Cf.* also DAHLBERG, 1947 a, p. 25 ff.). Table 7 may be of some use as a basis for the following deductions. It is presumed, as also in the following calculations, that males and females have the same frequency. In low frequencies the female heterozygotes are almost exactly twice as numerous as the defective males.

The effect of total selection may be calculated in two different ways. If only the male character-carriers (but all genes) are considered, which is quite justifiable in very low frequencies (where the female heterozygotes are infinitesimally few) but may be done up to a frequency of 1 per cent male character-carriers, the conditions are extremely simple. The frequency of the male character-carriers is unaltered in the first generation and in every following generation reduced exactly to half. The character and the gene (if not arisen anew through mutation) will be totally extinguished in a few generations. The formula for the reduction is extremely simple; if the male character-carriers have the frequency r, they have in the nth generation the frequency $\frac{r}{2^{n-1}}$. Table 8 is rather superfluous but is given for a comparison with the following calculations. The formula is valid for all frequencies if only the male character-carriers are eliminated, and the table could thus be extended to higher frequencies, but in such frequencies this assumption is of course very unnatural.

Where the female homozygotes are extremely rare or almost non-existent these calculations are quite sufficient and they are practically adequate in all low frequencies. From a theoretical point of view, however, the result of selection of character-carriers of both sexes must be considered, and this is of course necessary especially in frequencies where the female recessive homozygotes play a more or less important role. DAHLBERG (1947 a, p. 44) gives a formula, evidently for males, for

the frequency of the gene. This formula is, of course, correct (with the modification that n should be counted from Gen. I) but it is assumed that the generation in which the effect of the selection is first apparent is the third after selection sets in, *i. e.* Gen. II as the generations are indicated in the present paper. This is the case when only the male character-carriers are eliminated (Table 8); when all character-carriers, males and females, are eliminated, the frequency is reduced already in the first generation though very little or in an almost imperceptible degree in the lowest frequencies. To apply to all generations from the first one — which has, it is true, little importance — the formula should therefore be slightly modified. When written to apply to all generations the formula for the total frequency of R, which is $^2/_3$ of the frequency in the males, is (the immediate effect; *cf.* below)

$$r_n = \frac{2r}{2^n\,(r+1)} \cdot \frac{2}{3}.$$

The formula for the total frequency of the character-carriers (= frequency of R in the females = half the frequency of R in the males) is still simpler:

$$RO_n = \frac{r}{2^n\,(r+1)}.$$

The following calculations are mostly made not by means of this formula but directly. Principally the method of HOGBEN (1946, p. 130), which seems rather complicated but is in reality very simple, is used.

Table 9 shows for a number of frequencies the result (in panmixia) in the first two generations of elimination of the character-carriers, males and females. In the following generations, as in the second one, the frequency is always half of that in the foregoing. The female character-carriers are removed from the first generation and can, as far as total selection is going on, because of the mode of inheritance never arise anew.

A more perspicuous picture of the rules for the reduction is given in the diagrams Figs. 12 and 13, in which a comparison with total selection in monomeric autosomal recessivity is also presented. (The extreme case of 99 per cent character-carriers, all eliminated, is of course rather unnatural but elucidates the general principles.) In high frequencies there is a very steep fall of the curve from the initial to the first generation. The fall is gradually less and less steep. In the initial frequency of 1 per cent the frequency is very little reduced in the first generation; in considerably lower frequencies the reduction is almost

TABLE 9. *Sex-linked recessivity. Effect of elimination of character-carriers in two generations. Frequencies in per cent (♂ per cent of ♂♂, etc.).*

	0	I	II
Character-carriers	99	24,916	12,458
♂ *RO*	99,332	49,832	24,915
♀ *RR*	98,668	—	—
♀ *DR*	1,327	49,832	24,916
Character-carriers	64	21,214	10,607
♂ *RO*	73,693	42,428	21,214
♀ *RR*	54,307	—	—
♀ *DR*	38,773	42,428	21,214
Character-carriers	50	19,098	9,549
♂ *RO*	61,803	38,197	19,098
♀ *RR*	38,197	—	—
♀ *DR*	47,214	38,197	19,098
Character-carriers	37,5	16,667	8,333
♂ *RO*	50	33,333	16,667
♀ *RR*	25	—	—
♀ *DR*	50	33,333	16,667
Character-carriers	10	7,294	3,647
♂ *RO*	17,082	14,589	7,294
♀ *RR*	2,918	—	—
♀ *DR*	28,328	14,589	7,294
Character-carriers	4	3,4648	1,7324
♂ *RO*	7,4456	6,9297	3,4648
♀ *RR*	0,5544	—	—
♀ *DR*	13,7825	6,9297	3,4648
Character-carriers	1	0,9619	0,4809
♂ *RO*	1,9618	1,9238	0,9619
♀ *RR*	0,0385	—	—
♀ *DR*	3,8461	1,9238	0,9619
Character-carriers	0,5	0,4927	0,2464
♂ *RO*	0,9902	0,9855	0,4927
♀ *RR*	0,0098	—	—
♀ *DR*	1,9708	0,9855	0,4927
Character-carriers	0,1	0,0996	0,0498
♂ *RO*	0,1996	0,1992	0,0996
♀ *RR*	0,0004	—	—
♀ *DR*	0,3984	0,1992	0,0996
Character-carriers	0,02	0,019987	0,009993
♂ *RO*	0,039984	0,039973	0,019987
♀ *RR*	0,000016	—	—
♀ *DR*	0,079946	0,039973	0,019987

	0	I	II
Character-carriers	0,01	0,009996	0,004998
♂ *RO*	0,019996	0,019992	0,009996
♀ *RR*	0,000004	—	—
♀ *DR*	0,039984	0,019992	0,009996

imperceptible and practically begins after this generation. The differences are of course caused by the female homozygote character-carriers, which in lower frequencies play a more and more diminishing role and from about 0,1 per cent are practically almost non-existent.

In high frequencies the effect of selection is at first, at least after the first generation, a little less strong than in autosomal recessivity, but the frequencies are soon reversed and the curves cross (Fig. 12). In medium frequencies (10 per cent, Figs. 12 and 13) the reduction is from the beginning much stronger than in autosomal recessivity and the curves are strongly divergent. In lower frequencies (from 4 per cent;

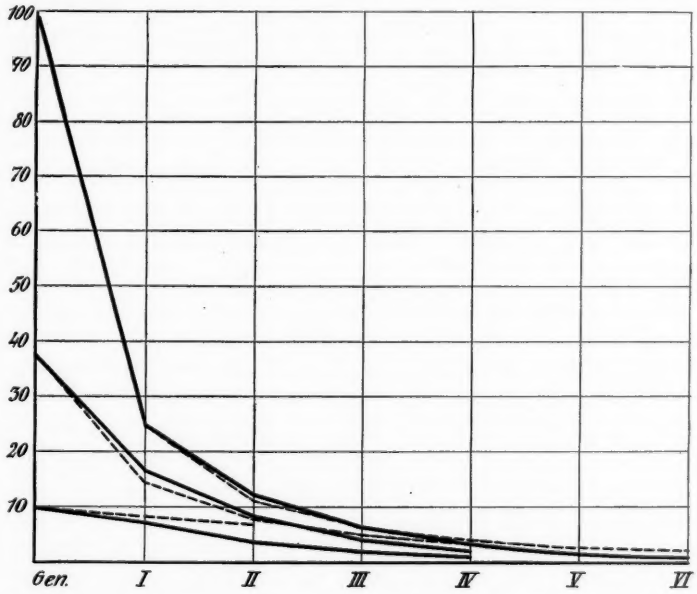

Fig. 12. Sex-linked recessivity. Total elimination of recessive character-carriers. Initial frequencies 99, 37,5 and 10 per cent. Dashed lines total elimination in autosomal recessivity. (To make the diagram clearer only the curves for 99 per cent are continued until Gen. VI.)

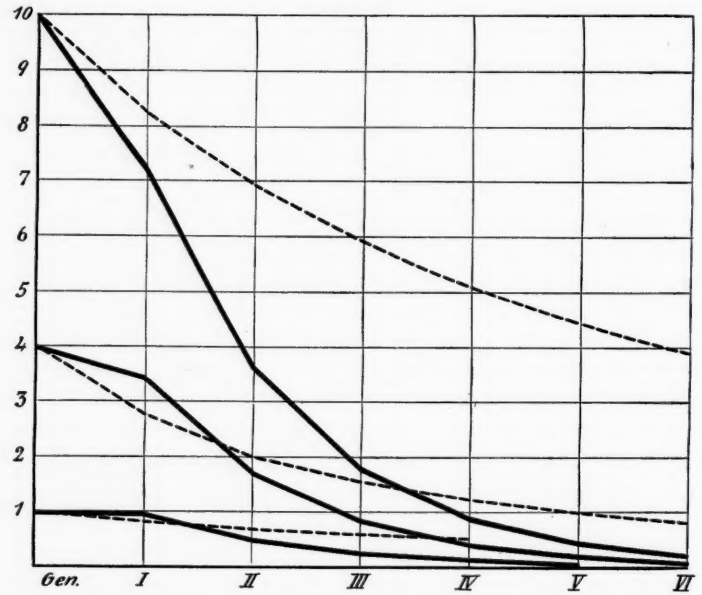

Fig. 13. Sex-linked recessivity. As Fig. 12. Initial frequencies 10, 4 and 1 per cent.

Fig. 13), owing to the small or insignificant reduction in the first generation, the reduction is in this generation stronger in autosomal recessivity but the frequencies are then reversed and the curves cross as in the highest frequencies.

All frequiencies calculated above and given in the tables and diagrams represent the *immediate* effect of a repeated elimination of the character-carriers. Initially, before selection sets in, there is equilibrium between the gene frequency in males and females. Through the first elimination this equilibrium is abolished. From the first generation there is a surplus of the gene in the males and a corresponding deficit in the females; as was pointed out before, the frequency in the latter is always exactly half of that in the males, and the total frequency, which in equilibrium would exist in both sexes, is $^2/_3$ of the frequency in the males. If elimination ceases, the difference is gradually levelled and equilibrium restored. This process is at first rather rapid and the difference is small from the fifth generation. Full equilibrium, however, is attained very slowly, after about 17 generations.

TABLE 10. *Sex-linked recessivity. Example of »equilibrization» after elimination of character-carriers. Cf. the text. Frequencies in per cent. Gen. 0 = Gen. II in Table 9.*

Gen.	♂ *R*	♀ *R*
0	16,67	8,33
I	8,33	12,5
II	12,5	10,417
III	10,417	11,458
IV	11,458	10,937
V	10,937	11,198
VI	11,198	11,068
VII	11,068	11,133
VIII	11,133	11,1003
IX	11,1003	11,1165
X	11,1165	11,1084
XI	11,1084	11,1124
XII	11,1124	11,1104
XIII	11,1104	11,1114
XIV	11,1114	11,1109
XV	11,1109	11,1112
XVI	11,1112	11,11107
XVII	11,11107	11,11113

The following example may be of some interest. If the dominant and the recessive gene have initially the same frequency, the population has in equilibrium the composition ♂♂ 2 *DO* : 2 *RO*, ♀♀ 1 *DD* : 2 *DR* : 1 *RR*. *R* are in both sexes $^1/_2 = 50$ per cent. After total elimination of *RO* and *RR* Gen. II has the composition ♂♂ 5 *DO* : 1 *RO*, ♀♀ 5 *DD* : 1 *DR*. ♂ *R* are $^1/_6 = 16{,}67$ per cent, ♀ *R* $^1/_{12} = 8{,}33$ per cent; the total frequency, which would exist in equilibrium, is $^1/_9 = 11{,}11$ per cent. If selection is stopped, panmixia gives the result shown in Table 10. As in other cases it is postulated that the generations are sharply limited — of course a fictitious assumption.

The table speaks for itself. The male gene frequency is, for obvious reasons, always that which represented female gene frequency in the preceding generation. The surplus of genes, under steady and gradually very slow reduction, is thrown to and fro between males and females until the surplus is infinitesimally small. From Gen. V. the difference is very small, from Gen. X or XI insignificant, but equilibrium is not quite established (perhaps not yet mathematically) until Gen. XVII.

The relation of the genotypes is altered from ♂♂ $^5/_6$ *DO* : $^1/_6$ *RO*, ♀♀ $^5/_6$ *DD* : $^1/_6$ *DR* to ♂♂ $^8/_9$ *DO* : $^1/_9$ *RO*, ♀♀ $^{64}/_{81}$ *DD* : $^{16}/_{81}$ *DR* : $^1/_{81}$ *RR* or in per cent from ♂♂ 83,33 *DO* : 16,67 *RO*, ♀♀ 83,33 *DD* : 16,67 *DR* to ♂♂ 89,89 *DO* : 11,11 *RO*, ♀♀ 79,01 *DD* : 19,75 *DR* : 1,23 *RR*.

The re-establishment of equilibrium has the same course in all frequencies.

SEX-LINKED RECESSIVITY. PARTIAL SELECTION.

Elimination of 0,5 of the character-carriers gives in high frequencies (64 per cent; Fig. 14) in several generations a reduction which is less strong than in autosomal recessivity, but the difference is not great. The curves are at first slowly divergent and then convergent. In the example they meet in Gen. X (frequency 4,67, in autosomal recessivity 4,6 per cent) and from then on diverge slowly; the frequency will approach zero very much sooner in sex-linked than in autosomal inheritance.

In low frequencies (4 and 1 per cent; Fig. 15) the reduction curve has the same general course as in total elimination. It falls slightly or almost not at all to Gen. I, still less than in total elimination (1 per cent to 0,98 per cent, *etc.*). From Gen. I to Gen. II it falls rather much, crossing the autosomal curve, then more slowly. The two curves diverge rapidly. When in sex-linked inheritance the frequency approaches zero (in an initial frequency of 1 per cent presumably somewhere between Gen. XX and Gen. XXX), the frequency in autosomal recessivity will still be relatively high.

As in total selection (p. 178) the equilibrium between the genes in the two sexes is abolished through the first elimination; there is a surplus in the males, a corresponding deficit in the females. Unlike that which is the case in total selection the relation between the genes is not constant but different in different initial frequencies and in different generations. What occurs is preferably illustrated by the diagram in Fig. 16 in which the curves, for one high and one lower frequency, indicate the frequency of the genes in females in per cent of their frequency in males.

In high frequencies (64 per cent in the diagram) the curve falls rather considerably to Gen. I; the first elimination, in other words, causes a relatively great difference between the sexes. It continues to fall, from Gen. I to Gen. II very slightly, then almost evenly to 83 per cent in Gen. X and then, if the calculation is continued, a further 2 per cent. In low frequencies (4 per cent in the diagram) the curve falls abruptly and very strongly to Gen. I, then alternatively rises and falls, from Gen. I to Gen. III rather strongly, to Gen. VII or in lower frequencies still further; it then falls very slowly to a limiting value which is about 81 per cent. The curve for 1 per cent goes below and close to that for 4 per cent, so close that it can hardly be drawn on the same diagram.

These characteristic features are due to the peculiarities of this mode of inheritance. The gene (and genotype) frequency in the males always coincides with the reduced female gene frequency in the preceding

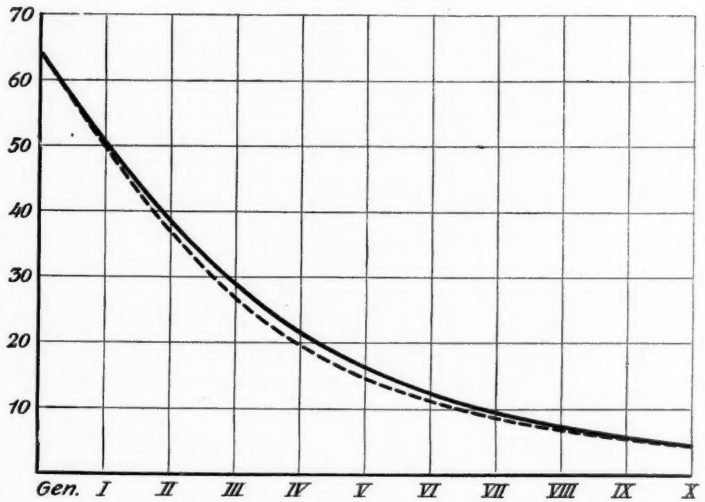

Fig. 14. Sex-linked recessivity. Elimination of 0,5 of the character-carriers. Initial frequency 64 per cent. Dashed line, the corresponding curve for autosomal recessivity.

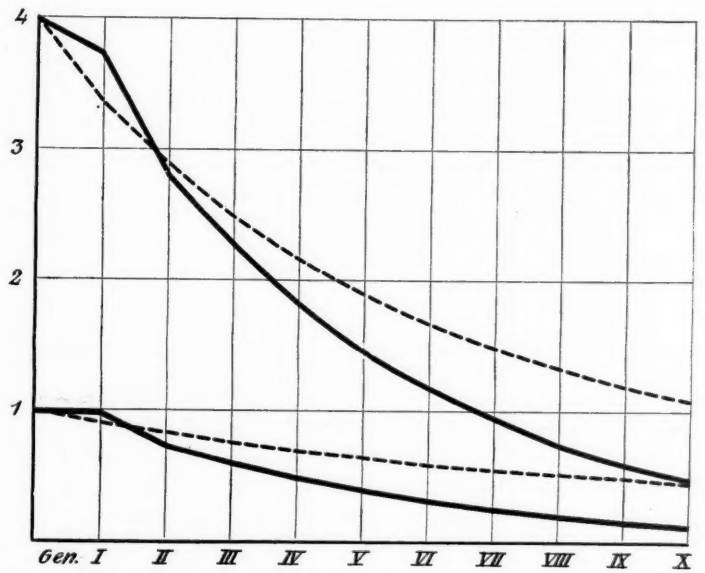

Fig. 15. Sex-linked recessivity. Elimination of 0,5 of the character-carriers. Initial frequencies 4 and 1 per cent. Dashed lines, autosomal recessivity.

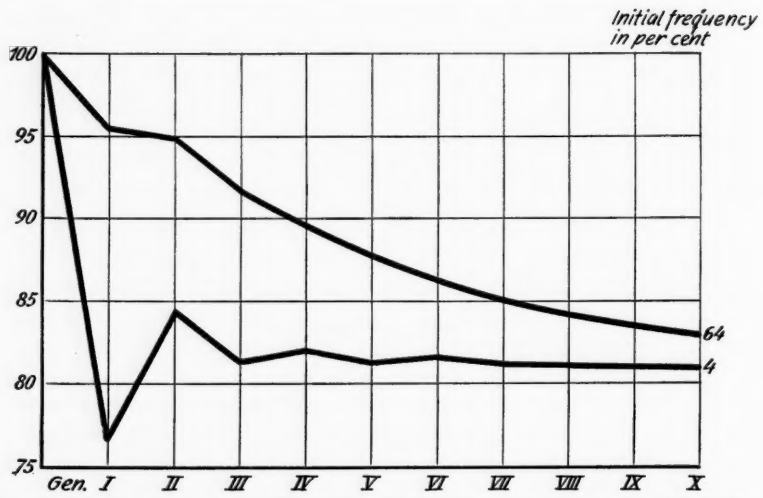

Fig. 16. Sex-linked recessivity. Elimination of 0,5 of the character-carriers. Frequency of the gene in the females in per cent of its frequency in the males. Initial frequencies 64 and 4 per cent.

generation (as before, sharply limited generations are assumed). In low frequencies the male gene frequency therefore is very little reduced, whereas the female gene frequency, influenced principally by the initial elimination of the males, is strongly diminished. What follows in the subsequent generations may be described as a wave-like after-effect and levelling of this primary effect of the partial selection.

If selection ceases, the difference between the sexes is slowly levelled. The principle is the same as in total selection (p. 178), and a special analysis may be omitted.

MONOMERIC AUTOSOMAL DOMINANCE. PARTIAL SELECTION.

In monomeric autosomal dominance total elimination of the character-carriers affords no theoretical problems, provided that the dominance is complete and the character developed before the age of reproduction (if not, the elimination is of course not total). The character and the gene will both disappear at once, and if they arise anew through mutation, they will always be immediately removed. What should be investigated and may have practical importance is the effect of partial elimination. As before, in the case of monomeric recessivity, I shall

TABLE 11. *Monomeric dominance. Effect in the first generation of elimination of 0,5 of the character-carriers. Frequencies in per cent. Bottom line frequencies in per cent of the initial frequency.*

Gen. 0	99	84	75	64	36	19	10	4	1	0,2	0,1
Gen. 1	98,81	76,69	64	50,17	22,90	10,74	5,33	2,05	0,503	0,1	0,05
Rel. freq.	99,81	91,3	85,33	78,4	63,61	56,55	53,29	51,275	50,31	50,06	50,0

dwell especially upon elimination of half of the dominant character-carriers (homozygotes plus heterozygotes).

Table 11 shows the reduction in the first generation. Instead of a comparison with the result in total elimination, which is of course impossible, the reduced frequencies are set in relation to the initial ones. In very high frequencies — where it would seem more appropriate to speak of an increased reproduction of the sparse recessive homozygotes

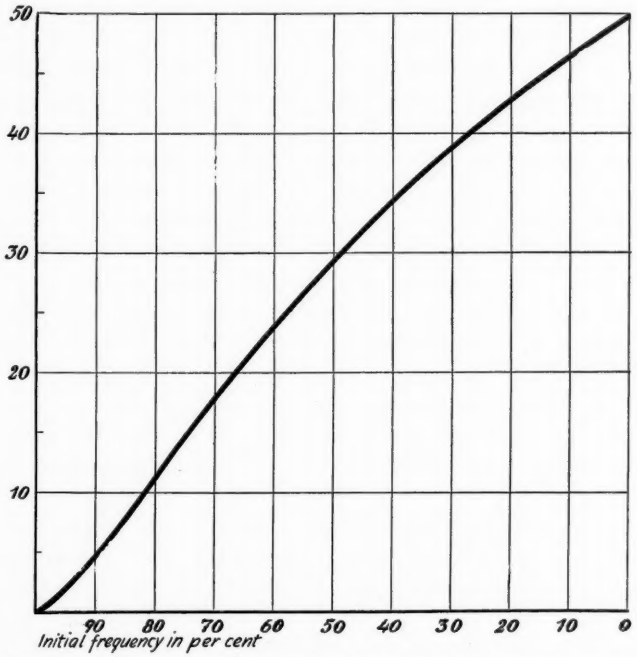

Fig. 17. Monomeric dominance. Elimination of 0,5 of the character-carriers. Reduction in the first generation: frequencies in per cent of the initial frequency.

TABLE 12. *Monomeric autosomal dominance. Elimination of 0,5 of the character-carriers. Frequencies in per cent. The figures on the second line for the different frequencies indicate the frequency in per cent of the initial frequency.*

0	I	II	III	IV	V	VI	VII	VIII	IX	X	XV	XX
99	98,81	98,58	98,27	97,85	97,30	96,52	95,43	93,84	91,62	87,60	33,80	1,75
—	99,81	99,57	99,26	98,84	98,28	97,50	96,39	94,79	92,58	88,48	34,13	1,76
64	50,17	35,41	22,44	12,98	7,05	3,69	1,89	0,96	0,48	0,24	—	—
—	78,40	55,32	35,06	20,28	11,02	5,76	2,94	1,49	0,75	0,38	—	—
4	2,05	1,04	0,523	0,262	0,128	0,064	0,032	0,016	—	—	—	—
—	51,27	25,97	13,07	6,55	3,2	1,6	0,8	0,4	—	—	—	—
1	0,503	0,252	0,126	0,063	(0,032)	(0,016)	—	—	—	—	—	—
—	50,31	25,2	12,6	6,3	(3,2)	(1,6)	—	—	—	—	—	—

— the reduction is exceedingly small. It rises with sinking frequency to the limiting value of 50 per cent. Already a frequency of 1 per cent is reduced almost to half. Fig. 17 shows that the curve for the reduction does not rise quite evenly to 50 per cent. It is at first (in the case of the highest frequencies) bent slightly downwards and then makes a feeble bow in the opposite direction.

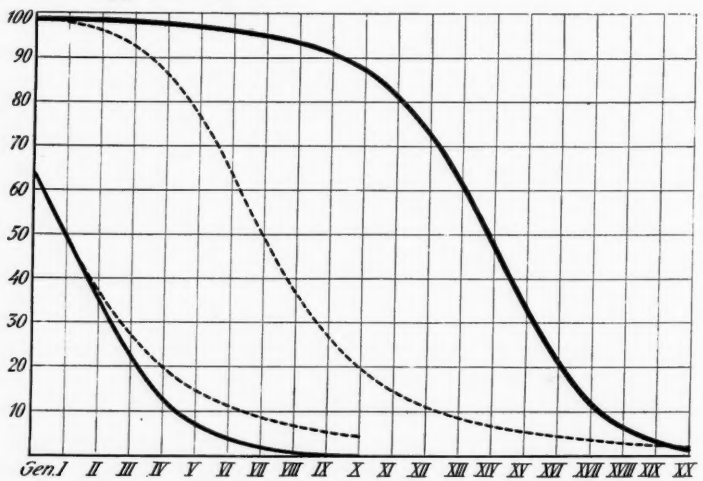

Fig. 18. Monomeric dominance. Elimination of 0,5 of the character-carriers. Initial frequencies 99 and 64 per cent. Dashed lines, the corresponding curves (0,5 elimination) for monomeric recessivity.

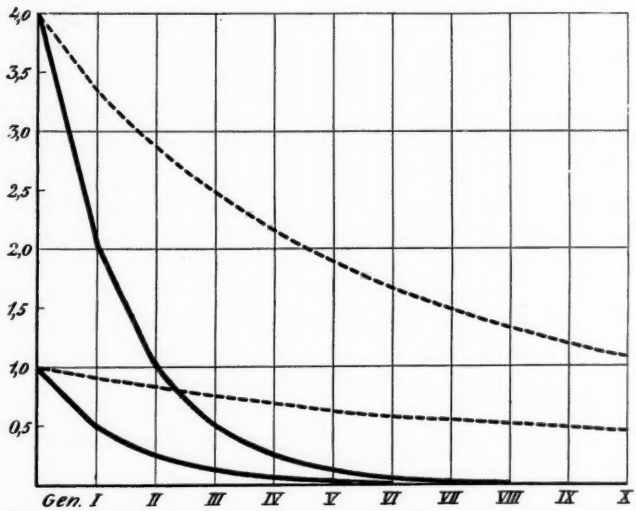

Fig. 19. Monomeric dominance. As Fig. 18 but initial frequencies 4 and 1 per cent.

Table 12 shows the reduction for four frequencies in 10 (in one case more) subsequent generations. A discussion is better appended to frequency curves.

The curve for the high initial frequency of 99 per cent (Fig. 18), though representing a very extreme case, is of considerable interest and contains the clue to the comprehension of all other cases. The frequency, of course, falls steadily but not evenly and the curve has (as in recessivity; *cf.* p. 168) the shape of a converted, very extended S. When the dominant character-carriers have from the beginning such a great predominance as in this example, even a reduction to half causes in the first generations only a slight change in the relative frequency of genes and genotypes. As the frequency of the dominant genes sinks, elimination of half of them has an ever increasing effect and the frequency curve falls more rapidly, more for every generation. This, however, occurs only to a certain point, in the example to Gen. XV and a frequency of about 34 per cent; from then on the curve bends slightly upwards in the opposite direction. This bending is the transition to the final stage where the frequency is approaching zero and the curve consequently is almost horizontal.

In lower frequencies the curve is practically coincident with the posterior part of the curve for 99 per cent or with its prolongation

(Fig. 19). The upward bending of the curve in the first generations is almost imperceptible from a frequency of about 64 per cent (Fig. 18); in lower frequencies the curve is from the beginning bent downwards (Fig. 19).

In consequence of the dominant inheritance even a partial elimination of this degree more or less rapidly leads to a strong diminution and soon extinction of the character-carriers (and the gene). From a frequency of about 4 per cent the frequency is in every subsequent generation reduced to about half. The low frequency 0,01 per cent (or a little less) is, in the examples given above, attained in the following generations: 99 per cent: Gen. XXXI; 64 per cent: Gen. XV; 4 per cent: Gen. IX; 1 per cent: Gen. VII.

A comparison with the effect of a selection of the same degree in monomeric recessivity is interesting. In the very high frequency of 99 per cent character-carriers the reduction is, for reasons sufficiently ex-

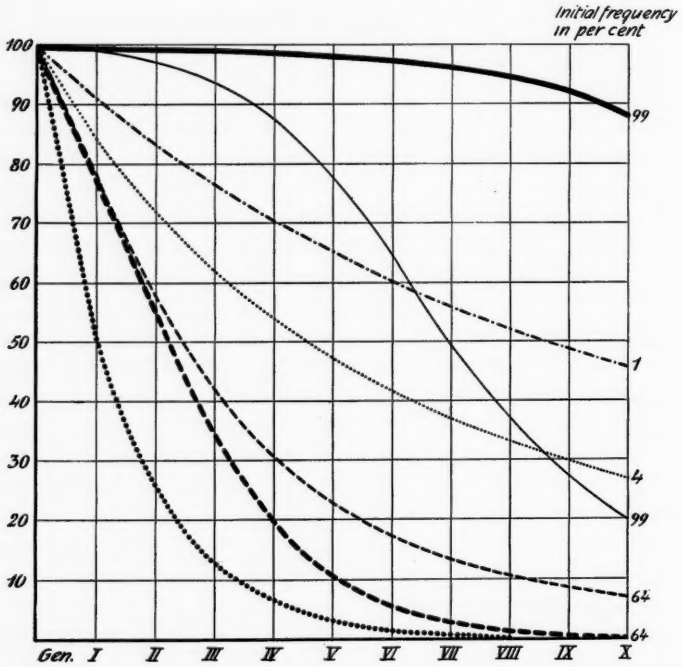

Fig. 20. Elimination of 0,5 of the character-carriers in monomeric dominance (thick lines) and monomeric (autosomal) recessivity (fine lines). Reduction in per cent of the initial frequencies. (Thick dotted curve, 4 per cent.)

plained above, in many generations much stronger in recessivity. The two curves are strongly divergent until Gen. X but from then convergent. They cross in (before) Gen. XX (Fig. 18). After a further ten generations the dominance curve, as stated above, approaches strongly zero, and the recessivity curve lies at about 0,8 per cent. From an initial frequency of about 64 per cent (where in the first generation the reduction is practically the same as in recessivity) the reduction is stronger in recessivity (Fig. 18). The curves are first divergent and later convergent but they never meet, because the dominance curve attains the zero line long before the point of intersection.

The differences between high and low frequencies and between dominance and recessivity are illustrated also by Fig. 20, where the curves for the relative, percentage reduction, *i. e.* from the same point of departure, are drawn. The dominance curve for 1 per cent would almost coincide with that for 4 per cent and is therefore not drawn.

Elimination of one tenth of the character-carriers has, of course, a much feebler effect. As shown by Table 13, which supplies the result for the first generation, the reduction is strongly increased with falling frequency. The limiting value, which in 50 per cent elimination is 50 per cent, is in 10 per cent elimination 90 per cent (*i. e.* a reduction of 10 per cent).

The reduction in subsequent generations is in very high frequencies extremely slow; a curve for 99 per cent is therefore not given. In an initial frequency of 64 per cent (Fig. 21) the reduction is in the first ten generations less strong than in the same initial frequency and monomeric recessivity, but the difference is not great. The curves are slightly divergent until Gen. VI and from then on convergent. They should soon meet and cross after Gen. XII.

The reduction is less strong than in recessivity until an initial frequency of 52 per cent. In lower frequencies the reduction is stronger than in recessivity and the frequency falls rapidly, more rapidly the lower the frequency. For an initial frequency of 1 per cent (Fig. 22)

TABLE 13. *As Table 11 but elimination of 0,1 of the dominant character-carriers.*

Gen. 0	99	64	50	36	10	4	1	0,1
Gen. I	98,98	62,13	47,58	33,86	9,11	3,62	0,90	0,09
Rel. freq.	99,98	97,08	95,17	94,05	91,13	90,45	90,11	90,01

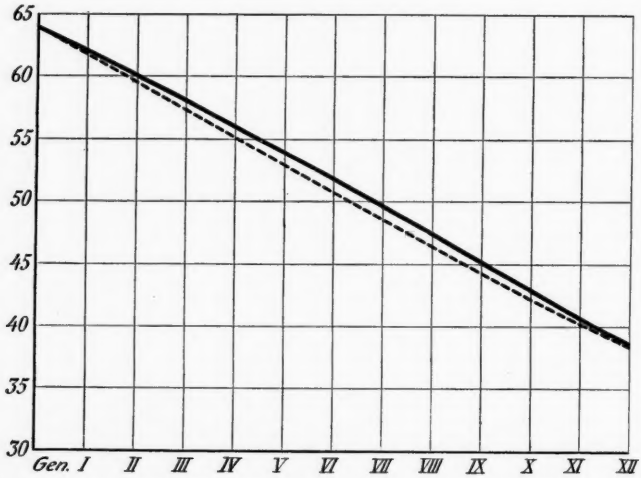

Fig. 21. Monomeric dominance. Elimination of 0,1 of the character-carriers. Initial frequency 64 per cent. Dashed line, the corresponding curve for monomeric recessivity.

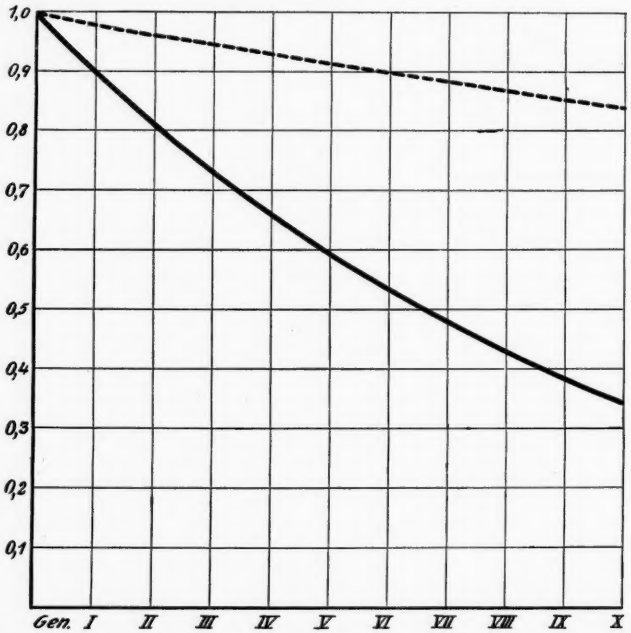

Fig. 22. Monomeric dominance. As Fig. 21 but initial frequency 1 per cent.

the curves are strongly divergent. The frequency is in dominance reduced in every generation to about 0,9 per cent and is already in Gen. VII brought down to less than half, at first sight a surprisingly rapid reduction considering the feeble selection. Extinction of the dominant gene, however, will require a great number of generations. A frequency of the character-carriers of 0,01 per cent, in 0,5 per cent elimination attained in Gen. VII, will presumably be attained towards about the 70th generation. In recessivity the frequency will then still be rather high, perhaps reduced at most to half.

SEX-LINKED DOMINANCE. PARTIAL SELECTION.

In sex-linked as well as in autosomal dominance total elimination of character-carriers of course causes immediate extinction of the gene and character. Partial elimination causes a reduction which is less strong than in autosomal dominance, though the difference is small or insignificant. The difference and its limitation are both due to the complex effect of the different mode of inheritance and the different gene frequencies which correspond to the same frequency of character-carriers. At the same gene frequency elimination causes a stronger reduction in sex-linkage; in the males, where genes and genotypes have the same relative frequency and are, in a way, equivalent, the gene frequency is reduced more than in the females, which have the same genotypical composition as the whole population in autosomal inheritance; a gene frequency of $^1/_2$, for instance, is by elimination of the character-carriers reduced (immediately, in the same generation) in the males to $^1/_3$, in the females to $^2/_5$. On the other hand, the same frequency of character-carriers is the result of a greater gene frequency in sex-linkage (75 per cent character-carriers, which in autosomal inheritance is the result of the gene frequency in the example given above, 50 per cent, corresponds in sex-linkage to a gene frequency of 63,4 per cent).

The features characteristic of partial selection in this mode of inheritance are most prominent in very high frequencies. Fig. 23 shows the result of 0,5 elimination in an initial frequency of the character-carriers of 95 per cent. The reduction curve has the same general shape as in autosomal dominance with a slight but distinct discrepancy in the first generations. The frequency sinks but little to Gen. I. The curve then sinks quite straight to (in this very frequency) Gen. III where an even bending begins. These features are due to the disparate genotypical structure of the males and are therefore more pronounced in the curve

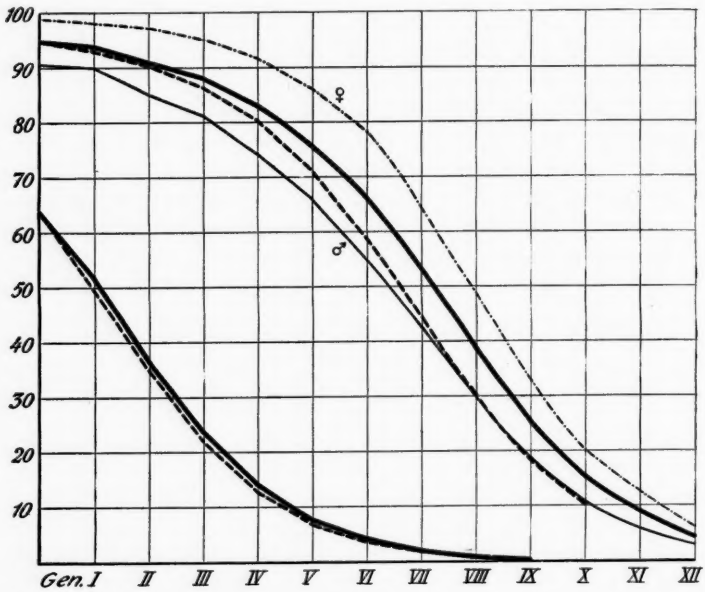

Fig. 23. Sex-linked dominance. Elimination of 0,5 of the character-carriers. Initial frequencies 95 and 64 per cent. Dashed lines, the corresponding curves for autosomal dominance. Fine lines, male and female character-carriers.

for this sex, which makes a very obtuse angle downwards between Gen. I and Gen. III (Fig. 23). The males always have the frequency of the female genes of the preceding generation after selection. The first elimination gives a very slight diminution of the female genes, which explains the feeble reduction of the males in Gen. I. The male genes are strongly diminished. This causes a strong reduction of the female character-carriers in Gen. I, whose frequency decides the frequency of the males in the next generation, *etc.*

The reduction is less strong than in autosomal dominance, where the male character-carriers consist of homo- and heterozygotes as well as the females, but the difference is not great. The curves diverge slowly until Gen. IX and then converge. When after a few generations the autosomal curve should approach zero, the distance between the two curves will be insignificant.

The effect of the partial selection increases with decreasing frequency and approaches more and more the effect in autosomal

dominance. Already for an initial frequency of 64 per cent, the curve still shows only a trace of the angle in Gen. I and is then bent downwards, going only a little above the autosomal curve (Fig. 23). The two curves are convergent and the character-carriers will be removed practically simultaneously; in Gen. IX the frequencies are about 0,55 and 0,50 per cent, respectively.

In an initial frequency of 4 per cent the reduction is practically though not mathematically the same as in autosomal dominance. A curve would coincide with the curve in Fig. 19 (in Gen. I a small difference would be barely discernible).

THE INFLUENCE OF INBREEDING (IN MONOMERIC AUTOSOMAL RECESSIVITY).

This chapter and the two subsequent logically should have followed after the sections dealing with autosomal recessivity. It seems preferable in one connection, however, to discuss the differences in the selection effect due to the mode of inheritance and then add analyses of the influence of deviations from panmixia, though these deviations are of importance chiefly in autosomal recessivity and will be discussed only so far as this mode of inheritance is concerned.

By inbreeding, the frequency of the homozygotes, as is well known, is augmented at the expense of the heterozygotes. This could possibly cause a stronger effect in the elimination of the recessive homozygotes. As DAHLBERG (1929 a, 1929 b, 1943, 1947 a) has shown, inbreeding in such degrees that can be imagined to occur in man could not cause an increase of the homozygotes which would appreciably change the composition of a population. The frequency of the recessive character-carriers, however, in low gene frequencies may be raised rather considerably, and it is of some theoretical interest to elucidate the subject from this point of view.

The first cousin marriage is the only inbreeding of importance in the case of man. Other consanguineous matings have almost no influence on the homozygosity and can be omitted. If the frequency of first cousin marriage is p, the increase of recessive homozygotes in a large population (where in panmixia such marriages would be extremely rare) is $\frac{p\,(r - r^2)}{16}$, and the frequency of the recessive homozygotes is $r^2 + \frac{p\,(r - r^2)}{16}$ (*cf.* DAHLBERG, 1929 a, p. 144; 1943, p. 76; 1947 a, p. 55). DAHLBERG has shown that the increase of the recessive homozygotes

TABLE 14. *Monomeric recessivity. Relative increase of the recessive homozygotes in extreme first cousin inbreeding (first cousin marriages only).*

Frequency of the recessive gene (*R*) in per cent	Frequency in panmixia of the recess. homozygotes (*RR*) in per cent	Frequency in extreme first cousin inbreeding of the recess. homozygotes in per cent	Increase of the recess. homozygotes in per cent of their panmixia frequency
90	81	81,56	0,69
80	64	65,0	1,56
70	49	50,31	2,68
60	36	37,50	4,17
50	25	26,56	6,25
40	16	17,50	9,37
30	9,0	10,31	14,55
20	4,0	5,0	25,0
15	2,25	3,047	35,4
10	1,0	1,5625	56,25
8	0,64	1,10	72
6	0,36	0,7125	98
5	0,25	0,547	115
4	0,16	0,40	150
3	0,09	0,272	202
2	0,04	0,1625	306
1	0,01	0,0719	619
0,5	0,0025	0,0326	1204
0,1	0,0001	0,0063	6244

attains its maximum at a frequency of the recessive gene of $^1/_2$ (50 per cent). In the extreme case of only first cousin marriages occurring ($p = 1$) the frequency of the recessive homozygotes is then increased from 25 to 25 + 1,562 per cent. The figure 1,562 falls uniformly with increasing and decreasing gene frequency (DAHLBERG, 1929 a, Fig. 5; 1943, Fig. 12; 1947 a, Fig. 12).

What is considered here is, however, the increase in relation to the entire population, and the matter should be regarded also from another point of view. If the *relative* increase of the homozygote frequency (*i. e.* the increase in relation to the frequency in panmixia) be regarded, it rises with decreasing gene frequency, as is shown by Table 14 and Fig. 24. From almost nil it rises to many hundred per cent and more of the panmixia frequency. Table 14 and Fig. 24 have, of course, no bearing on inbreeding in man but elucidate the general principle.

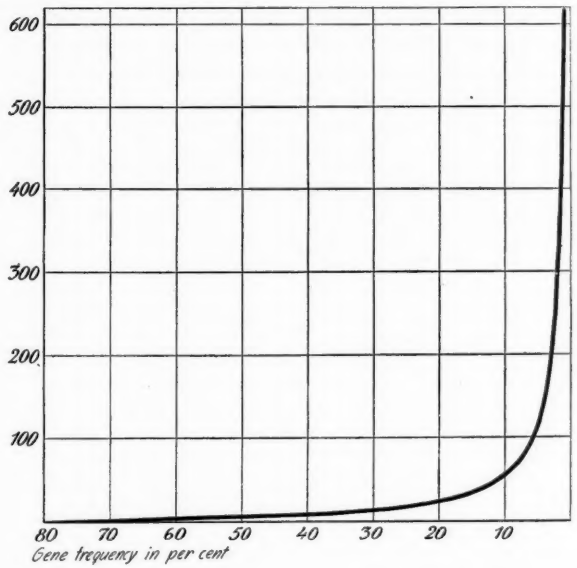

Fig. 24. Monomeric recessivity. Relative increase of the recessive homozygotes in extreme first cousin inbreeding (*cf.* Table 14). Gene frequencies between 80 and 1 per cent.

Random mating frequencies between about 0,1 and 0,0025 per cent are multiplied 3 to 13 times.

This high inbreeding would, if it were possible in man, have a marked influence on the effect of elimination of recessive character-carriers. At a cursory glance this influence may appear to be very strong in low frequencies. At a gene frequency of 1 per cent, for instance, the inbreeding frequency of *RR* is raised from 0,01 per cent in panmixia to 0,072 per cent or 7,2 in 10.000; after elimination of the recessive homozygotes in one generation only, it would, if after the elimination mating occurs at random and equilibrium is supposed to be established, be less than 1 in 10.000, whereas in panmixia the same frequency is reduced but to 6,8 in 10.000. This comparison, however, is wholly misleading, the strong decrease being mainly the result of the outspreading of the recessive genes through the assumed random mating. The comparison, therefore, should be made between cases with the same initial gene frequency (Table 15). In the example given above the gene frequency

TABLE 15. *Monomeric recessivity. Effect of elimination of recessive homozygotes in extreme first cousin inbreeding as compared with the effect in random mating. All figures in per cent.*

	Random mating			First cousin inbreeding		
R Initial frequency	*RR* Initial frequency	*R* Frequency after elimi-nat. of *RR* in 1 gener.	*RR* Frequency after elimi-nat. of *RR* in 1 gener.	*RR* Initial frequency	*R* Frequency after elimi-nat. of *RR* in 1 gener.	*RR* Panmixia freq. after elim. of *RR* in 1 gener.
50	25	33,333	11,111	26,56	31,915	10,186
20	4	16,667	2,778	5	15,789	2,493
10	1	9,091	0,826	1,56	8,571	0,735
4	0,16	3,846	0,148	0,40	3,614	0,131
1	0,010	0,990	0,0098	0,0719	0,9288	0,00863
0,5	0,0025	0,4975	0,00248	0,0336	0,4666	0,00218
0,1	0,0001	0,0999	0,0000998	0,0063	0,0937	0,0000877

1 per cent is in random mating in one generation decreased to 0,99, in first cousin inbreeding (where the homozygote frequency is raised from 0,01 to 0,07 per cent) to 0,93 per cent. The clearest conception of the difference is obtained, if the reduced »panmixia frequencies» in the inbred material, *i. e.* the frequencies obtained if panmixia and equilibrium are supposed to be established after the elimination (Table 15, last column), are compared with the corresponding figures in random mating.

Even in this extremely intense inbreeding elimination of the recessive homozygotes gives, as shown by the table, a reduction of their frequency which is stronger than in panmixia but not so much stronger than would be expected. The difference in comparison with the frequencies obtained in panmixia rises with sinking frequency of the gene but very slightly. At a gene frequency of 50 per cent the reduced frequency is 8,3 per cent lower than in panmixia, and this figure rises only to 12 per cent in the lowest frequencies. Yet the increase of the reduction would not be quite insignificant, if such an intense inbreeding were possible. While, for instance, 160 *RR* in 100.000 in random mating are diminished in one generation to about 150, the frequency would be reduced to 130. In very low frequencies the reduction would have but little practical importance. The bottom line in the table is, of course, intended only to illustrate the general principle.

Now, this whole discussion has, in a way, an unreal foundation, such exclusive first cousin mating not occurring in man nor in any other

TABLE 16. *Monomeric recessivity. Increase of the frequency of the recessive homozygotes by first cousin marriages of different degrees (in a large population).*

First cousin marriages, per cent	*R* per cent	*RR* in panmixia, per cent	*RR* increased frequency, per cent
10	10	1	1,056
5	10	1	1,028
1	10	1	1,0056
10	3,16	0,1	0,12
5	3,16	0,1	0,11
1	3,16	0,1	0,102
10	1	0,01 (100 in 1 million)	0,0162 (162 in 1 million)
5	1	0,01	0,0131 (131 » 1 »)
1	1	0,01	0,0106 (106 » 1 »)
10	0,32	0,001 (10 in 1 million)	0,003 (30 » 1 »)
5	0,32	0,001	0,002 (20 » 1 »)
1	0,32	0,001	0,0012 (12 » 1 »)
10	0,1	0,0001 (1 in 1 million)	0,0006 (6 » 1 »)
5	0,1	0,0001	0,0003 (3 » 1 »)
1	0,1	0,0001	0,000162 (1,6 » 1 »)

organism. The same general law is valid for lower grades of inbreeding, but has the effect any noteworthy importance? In Table 16 some examples are given of the increase in a large population of the frequency of recessive homozygotes, from 1 per cent downwards, obtained by first cousin marriages of a frequency between 1 and 10 per cent. It must be particularly emphasized that, as before, a large population without division in isolates is presumed (an unnatural presumption, as will be seen).

The increase of the frequency of the recessive homozygotes is, as in extreme first cousin mating, rising with sinking frequency of the gene. At 1 per cent first cousin marriages the increase is for 1 per cent *RR* 0,56, for 0,1 per cent *RR* 2, for 0,01 per cent *RR* 6,2, for 0,001 per cent *RR* 20 and for 0,0001 per cent *RR* 62 per cent of the panmixia frequency. At 5 and 10 per cent first cousin marriages the figures are multiplied five and ten times, respectively. The effect also rises regularly with intensification of the inbreeding. In a panmixia frequency of 1 per cent *RR* the increase of their frequency in 1, 5 and 10 per cent first cousin marriages is 0,56, 2,8 and 5,6 per cent, respectively. In a panmixia

frequency of 0,1 per cent *RR* the corresponding figures are 2, 10 and 20 per cent, *etc.*

All these figures have, as based on the presumption of intermarriage in a large population, a purely theoretical value. It is, however, of some interest to adopt this presumption for a while and consider the consequences. It is clearly seen that even for these conditions the consanguineous mating possible in man has no noteworthy influence on the composition of the whole population (as emphasized by DAHLBERG) and a small to moderate influence on the frequency of the recessive homozygotes. In large populations at the present time, first cousin marriages certainly never considerably exceed 1 per cent. An investigation of hospital patients in England gave 0,61 per cent (JULIA BELL, 1940), but the material may show an excess of consanguineous marriages (DAHLBERG, 1943, 1947 a). Figures from Prussia and Bavaria show a decrease, especially in the towns, from 0,7—0,9 per cent 1876—1880 to only 0,2 per cent. In France the frequency seems still to be or recently have been about 1 per cent (considerably lower in the towns than in the country districts). (*Cf.* DAHLBERG, 1938.) In most countries the frequency presumably is at most 0,5 per cent or more probably about 0,25 per cent. If the maximum value of 1 per cent is presumed, a panmixia *RR*-frequency of 1 per cent remains practically unaltered (Table 16); frequencies down to 0,01 per cent are almost unaltered or increased a few per cent. At very low frequencies of the recessive homozygotes the increase of their frequency is more noticeable (10 in a million raised to 12, *etc.*).

It is evident, too, that an inbreeding causing such a limited increase of the recessive homozygotes can have no great influence on the effect of elimination of these homozygotes. Nevertheless it is surprising to state, by means of an exact calculation, that in all frequencies of the recessive homozygotes the influence of 1 per cent first cousin mating is almost imperceptible, practically quite negligible (Table 17). The random mating frequencies are in the first generation decreased only by 0,1—0,125 per cent. The increase of the recessive homozygotes is, as seen before (Table 16), rising strongly with decreasing frequency (with 162 per cent in the theoretically possible frequency of 1 in a million), but this is almost completely (not quite) counterbalanced by the falling effect of the elimination. When this effect is exceedingly small or approaching zero, even a relatively great increase of the frequency makes no perceptible difference.

It is a fairly common belief that the more intense consanguineous

TABLE 17. *Effect of elimination of recessive homozygotes at 1 per cent first cousin marriages (in a large population) as compared with the effect in random mating.*

Initial frequency in per cent		Frequency of *RR* at 1 per cent first cousin marriages	Frequency of *RR* after elimination of *RR* in 1 generation	
R	*RR* random mating		Random mating	1 per cent first cousin marriages; panmixia frequency
50	25	25,0156	11,1111	11,1019
20	4	4,01	2,7778	2,7750
10	1	1,0056	0,82645	0,82551
3,16	0,1	0,102	0,09396	0,09385
1	0,01	0,0106	0,009803	0,009791
0,5	0,0025	0,002811	0,0024752	0,0024721
0,1	0,0001	0,000162	0,000099800	0,000099675

mating of older times, much more intense than what corresponds to 1 per cent first cousin marriages though, of course, not by far approaching 100 per cent, had a rather important influence on the frequency of rare recessive defects. It is often forgotten, then, that what causes the high alleged inbreeding in a small community is not a special inclination to marriages between relatives but merely the small size of the population. In a small primitive community the first cousin marriages, for instance, no doubt amount to 4—5 per cent, but they attain this high frequency mainly or exclusively through random mating. In a stationary population of 100 fertile people in the same generation (50 pairs, on an average 2 surviving children with the same fertility) the chance frequency of first cousin marriages, according to a formula of DAHLBERG, is about 4 per cent. In a population of 80 (40 pairs) the frequency is 5 per cent, in a population of 50 (25 pairs) it is 8 per cent (*cf.* DAHLBERG, 1929 a, p. 136 f.; 1943, p. 115 ff.; 1947 a, p. 91). If the average number of fertile children is 3 and the population, therefore, is rapidly increasing. the frequency is tripled. This intense consanguineous mating is, however, merely a consequence of random intermarriage and causes, therefore, no increase of the homozygote frequency in the small population. What is called inbreeding is, in this case, not inbreeding in the strict sense, which means a surplus of consanguineous mating above what results from panmixia. There is no reason for assuming that in a small community where mating at random gives, for instance, 5 per cent first cousin marriages, such marriages attain a higher (or note-

worthy higher) frequency. It is perhaps quite as probable that such marriages show a small, though doubtless a very small, deficit, many people even in primitive populations being unconsciously more inclined to marry other persons, second cousins, *etc.*, than their well-known first cousins. There are, it is true, other factors working in the opposite direction.

This trend of argument may be widened. The question arises if on the whole, except from special cases counterbalanced by others, a real inbreeding involving a noteworthy excess of consanguineous marriages occurs in human populations. The calculations of the effect of first cousin marriages given above are based on the assumption of mating, random except for the consanguineous marriages, in a large population but this assumption is an unnatural theoretical construction, the reproduction, as is well known, occurring in more or less sharply (or rather more or less diffusely) circumscribed *isolates*. The importance of this fact was first pointed out by WAHLUND (1928), who proposed the term *isolate* and analysed problems connected with this mode of reproduction. Now, a few decades ago 1 per cent first cousin marriages seem to have been a fairly normal frequency in rural populations, and such populations certainly still exist in many districts. This frequency of such marriages is a result of random mating in a stationary population (an isolate) of 400 individuals (200 pairs, *etc.*). The isolates now in view can hardly have been (and be, where they still exist) larger; 400 individuals seem to be a quite reasonable number. The decrease of the frequency of first cousin marriages rather plainly coincides with the growth and breaking up of the old country isolates.

DAHLBERG has pointed out (1943, p. 97, 159; 1947 a, p. 74, 130) that there will presumably be a surplus of first cousin marriages in populations where the marriage is arranged by the parents. Though no facts are available, I am inclined to believe that on the whole, and especially nowadays, this circumstance is of rather slight importance and is perhaps not increasing the homozygosity much more than it is decreased by the absence of marriages between siblings and between parents and children and the rarity of marriages uncle-niece and aunt-nephew. The fact that in French country districts the frequency of first cousin marriages has been markedly higher than 1 per cent (1,19 per cent 1898—1910) may point to some influence of the parental arrangement of the marriages, but the corresponding size of isolates is not much less than 350 individuals, and the increase of the percentage from 1,07 per cent 1898—1905 to 1,39 per cent 1906—1910 (statements for

a longer subsequent period are of course highly desirable), perhaps a result of a decrease of the isolates through removal to the towns as DAHLBERG suggests, points to the frequency of first cousin marriages even in France being mainly a result of random mating in isolates.

There are certainly particular cases of inbreeding and apparent inbreeding. In certain groups, such as jews, consanguineous marriages are no doubt much more common than in the average population. Such groups, however, from the reproductional view are isolates, though in our days usually not limited geographically. A genuine inbreeding may occur in certain families, as a rule in a few generations only, in royal families for longer periods. The well-known case of the Ptolemæans, often quoted in discussions of these problems, was unquestionably a case of true and intense inbreeding. In more recent times the royal families constitute (or have constituted) a social isolate (without importance, of course, for the composition of a whole population); in other cases it is, in a way, a matter of taste if we speak about a temporary inbreeding or about temporary isolates. At any rate, the homozygosity may be somewhat raised. Inquiries as to the occurrence and nature of such cases would, of course, be of great interest. They certainly play a rather insignificant role from the point of view of the population and are counterbalanced by others, where there is a deficit of consanguineous matings, and by the deficit of incestuous matings.

It may be objected that the argument given above is merely of a formal nature. Small communities may be characterized as »inbreeding areas»; consanguineous marriages unquestionably are much more common in them than in a mixed population. What constitutes genuine inbreeding, however, is not a high rate of consanguineous mating in itself but a *surplus* of such mating above what occurs in mating at random. All true inbreeding is principally of the same kind as the most intense form, that occurring in reproduction through repeated self-fertilization in hermaphroditic organisms.

Through splitting up of a population into isolates the rate of consanguineous mating is increased in the partial populations, consequently also in the whole of them. From the point of view of the entire population it could then be said that an inbreeding is going on. When the word is used by scientists acquainted with the genetics of human populations, as HALDANE and others, it is of course employed in this sense. But this is an inbreeding, it must be added, without the system of mating characteristic of true inbreeding, where relatives in ony way or another are preferred. It could be said that relatives are »preferred» in

relation to the entire population, because they must be, being in excess in the part of the population in which each individual has the possibility of marrying. There is, however, a fundamental difference between this excess of consanguineous matings and that caused through genuine inbreeding. If the genes are equally distributed, the surplus of consanguineous mating produces no increase whatever of the homozygosity, no more in the entire population than in the propagative circles. Reproduction in isolates, then, though involving a more or less important enhancement of the frequency of consanguineous matings both in the isolates and in the entire population, *in itself* causes no increase of the homozygosity. An equal distribution of all genes in a large population is no doubt inconceivable, but many not very rare genes may be fairly evenly spread. Anyway, from a logical point of view it is not appropriate and it will cause and has not unfrequently caused a good deal of confusion to employ the word inbreeding for a system of mating lacking in principle what is the most characteristic feature of true inbreeding.

Long after this chapter was written I have found that HOGBEN (1946) emphasizes that he uses the term inbreeding to signify a mating »which occurs when there is interbreeding between closely related individuals *in excess* of what would occur in a system of random mating» and that »this is not what is usually implied when people speak of an 'inbred' community».

Everything thus points to the frequency of consanguineous mating, whether low or high, being essentially a result of panmixia, *viz.* a function of the size of the isolates. DAHLBERG (1943, p. 80, 1947 a, p. 59) inclines towards the same view. »In many populations», he says, »marriages between cousins and other relations . . . can . . . be assumed to occur to about the same extent as is to be expected in panmixia, taking into account the size of the population». In the small communities of ancient times, where consanguineous marriages must have been very common and could be suspected, and have really been believed, to have caused a marked increase of the frequency of recessive defects, such defects undoubtedly were much more common than in modern populations, but this was due to a high inbreeding in the proper sense of the word but to the small size and fairly complete confinement of the isolates, in which rare recessive genes, different in different isolates, had attained a much higher frequency than in other parts of the population. This question will be treated under the following heading.

The conclusion, then, is that true inbreeding, which in any case

would have very little or almost no influence on the effect of elimination of recessive homozygotes, has in reality no such influence at all.

THE INFLUENCE OF ACCUMULATION IN ISOLATES.

If a gene causing a recessive defect (or other character) is very irregularly distributed and more or less strongly accumulated in certain isolates, which may be the case if it has arisen once by mutation and the frequency has been increased by chance in the isolates, or if it has been favoured by selection in the isolates or ill-favoured in the remaining population, the proportion between homo- and heterozygotes in the entire population is more or less severely disturbed and elimination of the character-carriers may have a stronger effect than in panmixia. Assume a gene *R* having a frequency of 1 per cent in a population (*r* 0,01). In panmixia *RR* are 0,01 per cent, *DR* 1,98 per cent, and an elimination of *RR* from reproduction has almost no effect (reduction from 10 to 9 in 100.000 in five generations; *cf.* Table 2). Let us now assume that *R* are all concentrated to an isolate embracing a tenth of the population. In this isolate *R* has then a frequency of 10 per cent; *RR* are 1 per cent and *DR* 18 per cent. This has two important consequences (*cf.* Fig. 25; I give this extremely simple diagram for readers which perhaps are not quite acquainted with these matters). The relation between homo- and heterozygotes is, if the frequency in the entire population is calculated, strongly altered; the heterozygotes are a little but not very much fewer than in random mating (1,8 instead of 1,98 per cent), the recessive homozygotes *RR* are ten times as many as in panmixia, *viz.* 0,1 per cent. The effect of elimination of the recessive character-carriers is entirely determined by the frequency relations within the isolate, where *RR*, as mentioned, are 1 per cent. A comparatively strong diminution occurs in the isolate, at complete elimination of the recessive homozygotes in one generation to 0,8, in two generations to 0,7 and in five to less than 0,5 per cent. This means that *RR* from 0,1 per cent of the population are reduced in one generation to 0,08, in two generations to 0,07 and in five to less than 0,05 per cent.

The isolate in this example is unnaturally great. The result is, however, the same if there are several isolates embracing together a tenth of the population. In Table 18 some examples are given of the effect of complete elimination of recessive homozygotes postulated to be entirely restricted to isolates embracing $^1/_2$, $^1/_{10}$, $^1/_{100}$, $^1/_{400}$, $^1/_{1000}$ and $^1/_{4000}$ of a population. By isolate here and in the following is meant the isolate or generally the total of the isolates in which *R* is assumed to occur. What

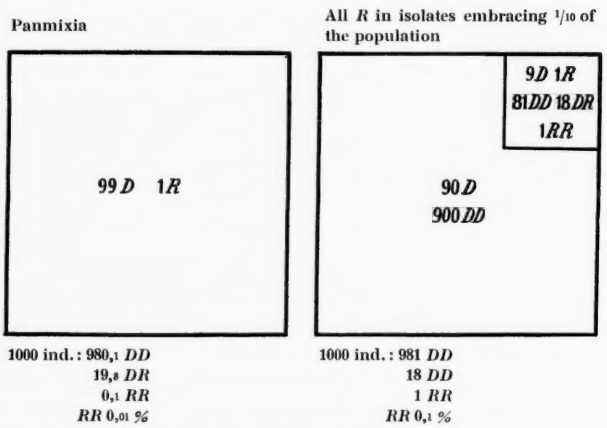

Fig. 25. Diagram illustrating the effect of complete confinement of a recessive gene in isolates.

is illustrated in the table is the reduction of the increased homozygote frequency in the population. The amount of the reduction is easily found by multiplying the reduced *RR*-frequency in the isolates, calculated in the usual way, by the size of the isolate(s).

The result depends on the size of the isolate (the total of isolates) and on the frequency of the gene. It is easily understood that a regular reciprocal dependency makes itself felt. If a gene is evenly distributed in all isolates, the result, as is easily understood, will be the same as in panmixia. Such cases, which are probably only theoretically possible, lead to others, in which a gene is present and has about the same frequency in a major part or a large number of the isolates constituting a population (in other words, in which a gene is absent only in a minor part of the population). The smaller the isolate (or the total of isolates) in which a recessive gene occurs, the stronger is the effect of the elimination of the recessive homozygotes compared with the effect in random mating (at the same gene frequency). — It goes without saying that the table and the arguments presented below are highly schematic in so far as it is presumed that a gene is totally confined to certain isolates and has the same frequency in all of them. It should be noticed, too, that several items of the table are rather preposterous from a practical point of view, especially the highest and lowest »initial frequencies» of the genes and recessive homozygotes; they have the purpose of elucidating the changes

TABLE 18. *Monomeric recessivity. Effect of accumulation in isolates.*

R per cent	Panmixia *RR* per cent	*R* all in (one or more) isolates						
			RR initial frequency in the isolate(s) per cent	*RR* initial frequency in per cent of the population	*RR* frequency in per cent of the population after elimination of *RR*. In brackets the corresponding figures at the same initial frequency of *RR* and panmixia			
					After 2 generations		After 5 generations	
10	1	isolate(s) 1/2 of the popul.	4	2	1	(1,2)	0,5	(0,7)
7,07	0,5		2	1	0,6	(0,7)	0,34	(0,44)
5	0,25		1	0,5	0,35	(0,38)	0,22	(0,27)
2,236	0,05		0,2	0,1	0,085	(0,088)	0,068	(0,075)
1	0,01		0,04	0,02	0,0185	(0,019)	0,0165	(0,017)
0,707	0,005		0,02	0,01	0,00945	(0,0096)	0,00868	(0,0091)
0,5	0,0025		0,01	0,005	0,00481	(0,00486)	0,00453	(0,00466)
0,2236	0,0005		0,002	0,001	0,000982	(0,00099)	0,000957	(0,00097)
0,1	0,0001		0,0004	0,0002	0,000198	(0,000199)	0,000196	(0,000197)
2	0,04	isolate(s) 1/10	4	0,4	0,2	(0,32)	0,1	(0,23)
1	0,01		1	0,1	0,07	(0,088)	0,044	(0,075)
0,632	0,004		0,4	0,04	0,0315	(0,037)	0,0231	(0,033)
0,316	0,001		0,1	0,01	0,0088	(0,0096)	0,0075	(0,0091)
0,1	0,0001		0,01	0,001	0,00096	(0,00099)	0,00091	(0,00097)
0,2	0,0004	isolate(s) 1/100	4	0,04	0,0204	(0,037)	0,01	(0,033)
0,1	0,0001		1	0,01	0,007	(0,0096)	0,0044	(0,0091)
0,0632	0,00004		0,4	0,004	0,00315	(0,0039)	0,00231	(0,0038)
0,0316	0,00001		0,1	0,001	0,000885	(0,00099)	0,000746	(0,00097)
0,01	0,000001		0,01	0,0001	0,000096	(0,0001)	0,000091	(0,000097)
0,05	0,000025	isolate(s) 1/400	4	0,01	0,0051	(0,0096)	0,0025	(0,0091)
0,0316	0,00001		1,6	0,004	0,0025	(0,0039)	0,0015	(0,0038)
0,0158	0,0000025		0,4	0,001	0,0008	(0,00099)	0,00058	(0,00097)
0,01	0,000001		0,16	0,0004	0,00034	(0,0004)	0,00028	(0,00039)
0,005	0,00000025		0,04	0,0001	0,0000944	(0,0001)	0,0000826	(0,000099)
0,02	0,000004	isolate(s) 1/1000	4	0,004	0,002	(0,0039)	0,001	(0,0038)
0,01	0,000001		1	0,001	0,0007	(0,00099)	0,00044	(0,00097)
0,00632	0,0000004		0,4	0,0004	0,000315	(0,0004)	0,000231	(0,00039)
0,00316	0,0000001		0,1	0,0001	0,000088	(0,0001)	0,000075	(0,000099)
0,002	0,00000004		0,04	0,00004	0,000037	(0,00004)	0,000033	(0,00004)
0,005	0,00000025	isol. 1/4000	4	0,001	0,00051	(0,00099)	0,00025	(0,00097)
0,00316	0,0000001		1,6	0,0004	0,00026	(0,0004)	0,00015	(0,00039)
0,00158	0,0000000025		0,4	0,0001	0,00008	(0,0001)	0,000057	(0,000099)
0,001	0,00000001		0,16	0,00004	0,000034	(0,00004)	0,000028	(0,00004)

TABLE 19. *Monomeric recessivity. R and RR in isolates. Initial frequency in the population 0,001 per cent. Frequency after elimination of RR in 2, 5 and 10 generations as compared with the corresponding frequencies in panmixia. (The figures for the reduction of the panmixia frequencies are approximate.)*

Size of the isolate(s)	Gen. II		Gen. V		Gen. X	
	Frequency in per cent	Reduction of the corresponding frequency in panmixia (in per cent)	Frequency in per cent	Reduction of the corresponding frequency in panmixia (in per cent)	Frequency in per cent	Reduction of the corresponding frequency in panmixia (in per cent)
1/2	0,000982	0,8	0,000957	1,36	0,000916	2,5
1/10	0,000961	2,9	0,000907	6,4	0,0008264	12,0
1/100	0,000885	10,7	0,000746	23,1	0,000577	38,6
1/400	0,000788	20,4	0,000577	40,5	0,000375	60,1
1/1000	0,000694	29,9	0,000444	54,2	0,000250	73,4
1/4000	0,000510	48,5	0,00025	74,2	0,000111	88,2

in the isolate effect in its dependence on gene frequency and size of isolate.

If the isolate is large, for instance $^1/_2$ of the population (of course many isolates), and the gene frequency is high, elimination has an effect that is markedly increased. (There are, as is well known, no monomeric recessive defects with such a frequency in man.) The increase of the effect decreases with decrease in the gene frequency and is in large isolates and low frequencies quite insignificant. What occurs is, in other words, a quite insignificant increase of an almost imperceptible reduction. On the other hand, the increase of the reduction is increasing with falling size of the isolates. This is seen in Table 18 and, for the initial frequency of 0,001 per cent *RR* (in the whole population) further elucidated by Table 19, in which the tenth generation also is included.

There is no reason to construct curves for the gradual reduction in all the examples in Table 18, but the curves given in Figs. 26—28 may have some interest. They concern isolates of $^1/_{10}$, $^1/_{100}$ and $^1/_{1000}$ and the same initial frequency, 1 per cent, of recessive homozygotes in the isolates; the curves for the reduced frequency consequently are coincident in the three cases, when the scale is the same. The figures illustrate clearly the increase of the reduction, in comparison with the reduction in panmixia, with decreasing size of the isolates.

As shown by the tables and Figs. 26—28, the isolate effect is in small isolates very strong. Where in panmixia elimination of the reces-

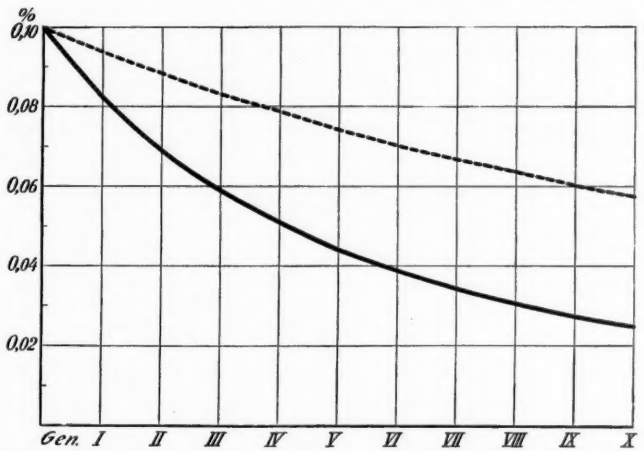

Fig. 26. Monomeric recessivity. Elimination of recessive homozygotes. Influence of accumulation in isolates. Isolates $^1/_{10}$, initial frequency in the population 0,1 per cent (in the isolates 1 per cent). Dashed line, reduction in panmixia.

sive homozygotes brings about almost no reduction, the frequency may be reduced in five generations until a fourth and in ten until a tenth.

It must be noticed, however, that the smaller the isolates, the smaller is the frequency in relation to the population, if a reasonable

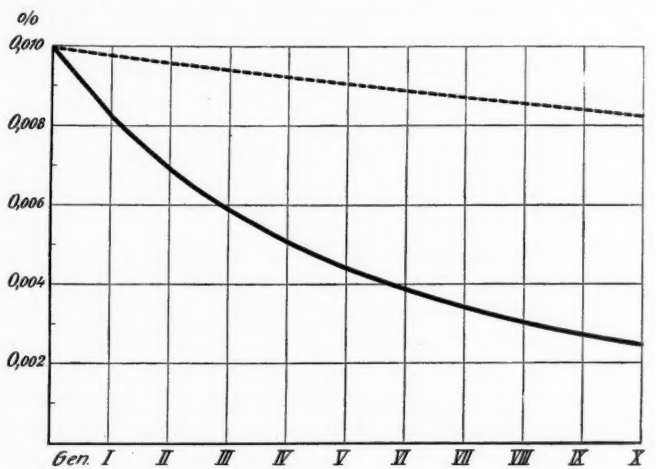

Fig. 27. As Fig. 26 but isolates $^1/_{100}$, initial frequency in the population 0,01 per cent.

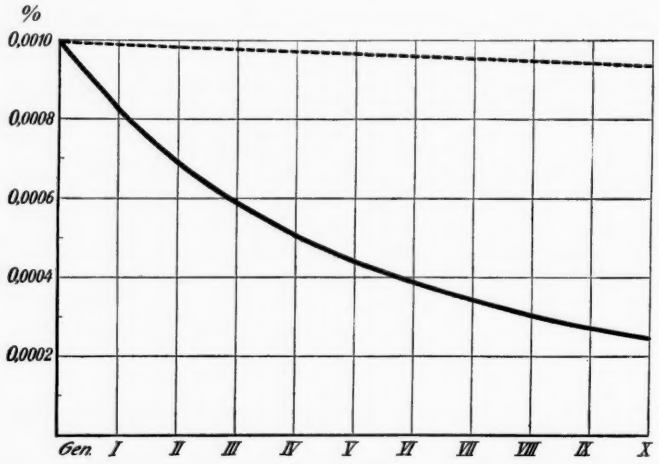

Fig. 28. As Fig. 26 but isolates $^1/_{1000}$, initial frequency in the population 0,001 per cent.

frequency in the isolates is assumed; the smaller is, consequently, also the panmixia reduction which is increased. When the frequency in the population as in several of the examples is but 1—4 in 1 million, *i. e.* is approaching zero, a reduction to, for instance, three fourth or the half means something from the point of view of the isolates but next to nothing from the point of view of the whole population. From this latter point of view the isolate effect has importance merely in cases of medium-sized isolates ($^1/_{100}$—$^1/_{1000}$) and a relatively high *RR*-frequency in the isolates (0,4—1,6 per cent in Table 18).

As seen in the tables, especially in Table 19, the increase of the effect of elimination is greater for every generation. In the long run the isolate effect will, therefore, have a certain importance even in cases where it is rather small in the first generations.

What is discussed so far and illustrated by Tables 18 and 19 is the effect of elimination on the increased frequency. The recessive homozygotes are assumed to remain in the isolates and there is, therefore, a surplus of them in the population after the elimination as well as before. No direct information is obtained about the decrease of the genes. A conception of this decrease is obtained if the panmixia frequencies before and after the elimination are compared. The latter are easily calculated. If *R* in the isolates has the frequency r_i and s is the size of the isolate(s), the reduced frequency in the nth generation is

TABLE 20. *Monomeric recessivity. Effect of accumulation in isolates.*

Panmixia *RR* per cent		*R* all in (one or more) isolates. Elimination of *RR*. Panmixia frequency (isolates broken up, equilibrium established) after elimination in		Corresponding frequencies after elimination in panmixia in	
		2 generations	5 generations	2 generations	5 generations
1	isolate(s) $^1/_2$ of the popul.	0,510	0,250	0,694	0,444
0,5		0,304	0,172	0,384	0,273
0,25		0,174	0,111	0,207	0,160
0,05		0,0421	0,0334	0,0458	0,0405
0,01		0,00924	0,00826	0,00961	0,00907
0,005		0,00473	0,00436	0,00486	0,00466
0,0025		0,00240	0,00227	0,00245	0,00238
0,0005		0,000491	0,000478	0,000496	0,000489
0,0001		0,0000992	0,000980	0,0000996	0,0000490
0,04	isolate(s) $^1/_{10}$	0,0204	0,0100	0,0370	0,0331
0,01		0,0069	0,0044	0,0096	0,0091
0,004		0,00315	0,00231	0,00390	0,00376
0,001		0,000885	0,00746	0,000987	0,000969
0,0001		0,0000961	0,000907	0,0000996	0,0000991
0,0004	isolate(s) $^1/_{100}$	0,000204	0,000100	0,000397	0,000393
0,0001		0,000069	0,000044	0,0000996	0,0000991
0,00004		0,0000315	0,0000231	0,0000399	0,0000397
0,00001		0,00000885	0,00000746	0,00000999	0,00000997
0,000001		0,00000096	0,00000091	—	0,000000999
0,000025	isolate(s) $^1/_{400}$	0,00001275	0,00000625	0,0000249	0,0000247
0,00001		0,00000637	0,00000375	0,00000999	0,00000997
0,0000025		0,00000197	0,00000144	—	0,000002496
0,000001		0,00000086	0,00000069	—	0,000000999
0,00000025		0,00000023	0,00000021	—	—
0,000004	isolate(s) $^1/_{1000}$	0,00000204	0,00000100	—	0,00000399
0,000001		0,00000069	0,00000044	—	0,000000999
0,0000004		0,000000315	0,000000231	—	—
0,0000001		0,000000088	0,000000075	—	—
0,00000004		0,000000037	0,000000033	—	—
0,00000025	isolat. $^1/_{4000}$	0,0000001275	0,0000000625	—	—
0,0000001		0,0000000637	0,0000000375	—	—
0,000000025		0,0000000197	0,0000000144	—	—
0,00000001		0,0000000086	0,0000000069	—	—

$$r_n^2 = \left(\frac{r_i \cdot s}{1 + n \cdot r_i}\right)^2 \text{ or } \frac{r_i^2 \cdot s^2}{(1 + n \cdot r_i)^2}.$$

Table 20, for the same panmixia frequencies as Tables 18 and 19,

TABLE 21. *Monomeric recessivity. Influence of accumulation in isolates.*

Panmixia *RR* per cent		Elimination of *RR*. Reduction of the initial frequency in per cent			
		Generation II		Generation V	
		Isolates	Panmixia	Isolates	Panmixia
1	isolate(s) 1/2 of the popul.	49,0	30,6	75,0	55,6
0,5		39,2	23,2	65,6	45,4
0,25		30,6	17,2	55,6	36.0
0,05		15,8	8,4	33,2	19,0
0,01		7,4	3,9	17,4	9,3
0,005		5,4	2,8	12,8	6,8
0,0025		4,0	2,0	9,3	4,8
0,0005		1,8	0,9	4,3	2,2
0,0001		0,8	0,4	2,0	0,9
0,04	isol. 1/10	49,0	7,5	75,0	17,2
0,01		30,6	4,0	55,6	9,0
0,004		21,2	2,5	42,3	6,0
0,001		11,5	1,3	25,4	3,1
0,0001		3,9	0,4	9,3	0,9
0,0004	isol. 1/100	49,0	0,75	75,0	1,75
0,0001		30,6	0,4	55,6	0,9
0,00004		21,2	0,25	42,3	0,75
0,00001		11,5	0,1	25,4	0,3
0,000001		3,9	—	9,3	0,1
0,000025	isol. 1/400	49,0	0,2	75,0	0,4
0,00001		36,3	0,1	62,5	0,3
0,0000025		21,2	—	42,3	0,16
0,000001		14,3	—	30,6	0,1
0,00000025		7,6	—	17,4	—
0,000004	isol. 1/1000	49,0	0,2	75,0	0,2
0,000001		30,6	—	55,6	0,1
0,0000004		21,2	—	42,3	—
0,0000001		11,5	—	25,4	—
0,00000004		3,9	—	9,3	—
0,00000025	isol. 1/4000	49,0	—	75,0	—
0,0000001		36,3	—	62,5	—
0,000000025		21,2	—	42,3	—
0,00000001		14,3	—	30,6	—

is of limited interest but having calculated the figures I give them. When in panmixia the reduction is below 0,1 per cent, the figures are not given.

The increase of the effect of selection is, when the subject is con-

sidered from this point of view, not diminishing but augmenting with falling gene frequency. In isolates of $^1/_2$ the reduction is, for the series of frequencies given in Tables 20 and 21, augmenting from about 1,3 to 2 times the reduction in panmixia. In isolates of $^1/_{10}$ and $^1/_{100}$ the corresponding augmentation is from 4,6 to 10,3 and from 43 to 93 times. For the same initial frequencies the increase of the reduction is augmented very strongly with falling size of the isolates. The reduction of 0,0001 per cent, for instance, is in isolates of $^1/_2$, $^1/_{10}$ and $^1/_{1000}$ in two generations 2, 10 and 90 times the reduction in panmixia.

The smaller the isolates and the lower the frequency, the stronger is consequently the influence of the propagation in isolates. In the lowest frequencies even a strong reduction of the relative frequency has, of course, a mere theoretical interest. A reduction of the theoretical frequency from 0,00001 per cent, *i. e.* 1 in 10 million, to about 0,6 and 0,4 in 10 million and from 0,000001 per cent, *i. e.* 1 in 100 million or practically zero, to about 0,7 and 0,4 in 100 million has no importance whatever for the population. It may seem that high up in the tables the frequencies and the calculated reductions have no real interest. A panmixia frequency of 0,0001 per cent is only 1 individual in 1 million. What matters is, however, not the imaginary frequency that would occur in panmixia but the actual frequency brought about through the propagation in isolates (Table 18). The reduced frequencies in Table 20 are the frequencies in Table 18 converted into their panmixia values. As seen from Tables 18 and 19, a relatively marked increase of the effect of elimination of recessive homozygotes is in many cases possible, at least theoretically.

Practically speaking, the chances for a considerable influence of the reproduction in isolates are more limited, and this from two quite different causes: in the case of comparatively common genes because they are more or less wide-spread in the entire population and not sufficiently accumulated in isolates, in the case of very rare genes because the effect of elimination even at such an accumulation is insignificant. If a recessive gene has a frequency in the population of 0,01 to about 1 per cent and, consequently, the recessive homozygotes have a frequency of 0,000001 to about 0,01 per cent, a somewhat stronger increase of the effect may, however, occur under certain circumstances.

A fairly concrete example may be interesting. Juvenile amaurotic idiotia, according to the important researches of T. SJÖGREN, has in the Swedish population a genetical frequency (chance of disease) of 0,0038 per cent and shows an accumulation in 23 areas. In order to simplify

the calculations we assume a frequency of 0,004 per cent and 25 isolates. If the recessive gene is entirely limited (which it is not) to 25 isolates embracing $^1/_{400}$ of the population, which gives a reasonable size of the isolates (about 600 individuals of all ages in the period concerned in SJÖGREN's investigation), the recessive homozygotes *RR* have in the isolates a genetical frequency of 1,6 per cent. The heterozygotes are in the isolates 22,1 per cent, as related to the entire population 0,055 per cent. (SJÖGREN gives 1,2 per cent; he asserts that this figure holds good for panmixia only, but DAHLBERG's remark that it is totally fictitious is obviously legitimate.) The frequency of the gene in the population is 0,0316 per cent, and the recessive homozygotes (the amaurotic idiots) would in random mating be infinitely few, about 1 in 10 million births. The defect homozygotes, as is well known, never attain the age of procreation but are all eliminated. This complete selection would, under the supposition made above, cause a considerable and rather rapid decrease in frequency: within the isolates from 1,6 to 1 per cent in two and to 0,6 per cent in five generations; in the whole population the frequency would sink from 0,004 to 0,0025 and 0,0015 per cent, respectively (*cf.* Table 18). The panmixia frequency would sink from 0,00001 to 0,0000064 and 0,0000037 per cent, respectively (Table 20). After 10 generations the frequency would be very low: in the isolates 0,31 per cent, in the whole population 0,00673 per cent, panmixia frequency 0,0000019 per cent.

These estimations seem rather paradoxical; DAHLBERG relishes in quoting just amaurotic idiotia as an evidence for the inefficiency of sterilization against rare recessive defects. The inconsistency, to a large extent, is due to the fact that the suppositions made above are deliberately extremely simplified and not realized in reality; the gene no doubt is not entirely concentrated in the isolates, and these are no sharply limited circuits of propagation. Nevertheless, the investigation of SJÖGREN incontestably indicates such an accumulation, at least in ancient times, that this explanation cannot give the whole truth. If we, without altering the remaining suppositions (0,004 per cent *RR*, *etc.*) quite deliberately assume that half of the recessive homozygotes are in the isolates, half in the remaining population, their elimination from reproduction would cause a decrease in two generations to 0,0034, in five generations to 0,0029 and in ten generations to 0,00244 per cent. Under all circumstances a decrease of the frequency of the disease, not very rapid but yet relatively efficacious, must indisputably take place.

It has, as a matter of fact, never been proved that amaurotic idiotia

has not at one time been more common but later been diminished somewhat through natural sterility. SJÖGREN has proved a marked decrease in the frequency from the year 1917 and sees the cause in a breaking up of the isolates, perhaps also in the sinking nativity. This explanation is no doubt right; this rapid decrease cannot to an essential degree be due to the sterility of the defectives. The older statistics show an increase but give no information as to the real frequency, which may as well have undergone a slight diminution as it may have remained constant in relation to the size of the population or increased. In any case, even if the frequency of the disease has possibly undergone a slight reduction, this is obviously quite insufficient. The frequency of the defect should have been reduced long ago to about the same minimum in the isolates as it has now in the population. The inevitable conclusion is that a slight diminution caused by sterility is counterbalanced by an increase of the genes and homozygotes through mutation. At any rate, according to the modern conception of genetics and evolution, a certain formation of the deleterious gene through mutation must be presumed.

I return to the general discussion of the problem. In rural areas there are no doubt still many isolates of a very moderate size, in Sweden embracing about $^1/_{4000}$ of the population. (Such an isolate would embrace about 1700 individuals of all ages and generations.) DAHLBERG is no doubt right in assuming a much greater average size in our days but this does not hinder that isolates of the said size are still comparatively common; I am convinced that there are in several country-districts a not quite negligible number of still smaller isolates. Such isolates and the smaller ones of past times are of special interest in regard to the origin of new genes through mutation. If a recessive gene causing a defect originates by mutation, it may in some generations in an isolate of this order of magnitude attain a frequency of perhaps 4 per cent (the last example in Table 18), which means 7,7 per cent heterozygotes and 0,16 per cent defective homozygotes. Dispersed in the entire population these genes would give a *RR*-frequency of practically zero, although of course defective individuals could occasionally originate in cases of consanguineous marriages. A reduction of the frequency of the genes by elimination of character-carriers would be wholly excluded. If, however, the genes remain in the isolate (or the isolates), elimination of the defective individuals from propagation would cause a diminution which would not be very great but yet worthy of consideration: from 1,6 defects in 1000 in the isolate to 1,4 in two and to 1,1 in five generations. Even in relation to the whole population there would be a per-

ceivable diminution, although of theoretical interest only if a single isolate be concerned (in the example of Table 18 from 4 to 3 in 10 millions in five generations). The result is, however, the same if the processes (mutation, accidental increase of the gene frequency, elimination) are going on in several isolates together having a larger size (*cf.* Table 18).

The problem of mutation will be discussed later on from a more general point of view.

I am far from considering that the facts and inferences presented above have any great practical importance for human populations. If the isolates commonly were still as small and secluded as in ancient times, the effect of elimination of rare recessive character-carriers would be markedly increased; in modern populations a small increase of the effect is theoretically possible but will hardly have any noteworthy practical importance. The chief interest is confined to cases of natural sterility, complete or partial, but even in those the breaking up of the isolates greatly reduces their influence.

From a general biological point of view even a quite insignificant effect of selection, without interest for a reasonably near future of mankind, has, of course, an incontestable interest.

THE INFLUENCE OF ASSORTATIVE MATING.

Assortative mating, where character-carriers tend to mate with their own kind in larger proportion than when mating occurs at random (positive assortative mating), increases the frequency of homozygotes at the expense of the heterozygotes and thus increases the effect of elimination of the character-carriers. (I use the term assortative mating in the usual sense, not in the wider sense of HOGBEN.) If the assortative mating is complete, character-carriers mating exclusively among themselves, which of course is quite impossible in man, the homozygotes increase rapidly in number and the heterozygotes are finally altogether exterminated. In monomerism, if the recessive gene has a frequency of 10 per cent, which at random mating gives 1 per cent recessive homozygotes, the frequency of these is increased after 100 generations to 9,2 per cent and finally (after more than 1000 generations, it is true) to 10 per cent; the heterozygotes are then entirely eradicated (DAHLBERG, 1943 and 1947 a, Table 10). Elimination of the recessive homozygotes in such cases would obviously have an immediate and maximal effect.

What may occur in man, and to a certain extent certainly occurs, is a partial assortative mating. The effect of this process on the genetical

TABLE 22. *Monomeric recessivity. Assortative mating, k 0,30. Effect of total elimination of RR compared with the effect in panmixia. All figures in per cent.*

	0		I		II		III		IV		V	
	R	*RR*	*R*	*RR*	*R*	*RR*	*R*	*RR*	*R*	*RR*	*R*	*RR*
Panmixia	10	1	9,09	0,826	8,33	0,694	7,69	0,592	7,14	0,510	6,66	0,444
	6,86	0,470	6,42	0,412	6,02	0,363	5,68	0,323	5,44	0,296	5,11	0,260
Assort. mating *k* 0,30	6,86	1	5,92	0,350	5,59	0,312	5,29	0,280	5,03	0,253	4,79	0,229

VI		VII		VIII		IX		X	
R	*RR*	*R*	*RR*	*R*	*RR*	*R*	*RR*	*R*	*RR*
6,25	0,391	5,88	0,346	5,56	0,309	5,26	0,277	5,0	0,250
4,86	0,236	4,64	0,215	4,43	0,196	4,24	0,180	4,06	0,165
4,57	0,209	4,37	0,191	4,24	0,175	4,02	0,161	3,86	0,149

structure of the population has been analysed in a very perspicuous way by DAHLBERG (1943, 1947 a). He infers that even a very strong assortative mating has but a limited effect on the relation between homo- and heterozygotes; the effect is stronger on rare characters but never very great. What we have to consider in this connection is the influence on the effect of the elimination of recessive character-carriers.

We assume at first that the assortative mating has the very high intensity of 30 per cent (k in DAHLBERG's formula and tables $= 0{,}30$), *i. e.* that 60 per cent of the matings between character-carriers and other individuals are not realized. If the recessive gene has a frequency of 10 per cent and consequently the recessive homozygotes initially, in random mating, were 1 per cent, their frequency is (after seven generations) raised to 1,98 per cent (DAHLBERG's Table 12); the heterozygotes are decreased from 18 to 16,14 per cent. Elimination of the recessive homozygotes takes in almost twice as many individuals and genes than in the case of random mating, which must cause a rather great increase of the effect.

To understand the result a more thorough comparison must be made. I prefer here to choose an example where the recessive homozygotes have a frequency of 1 per cent not in random mating but in

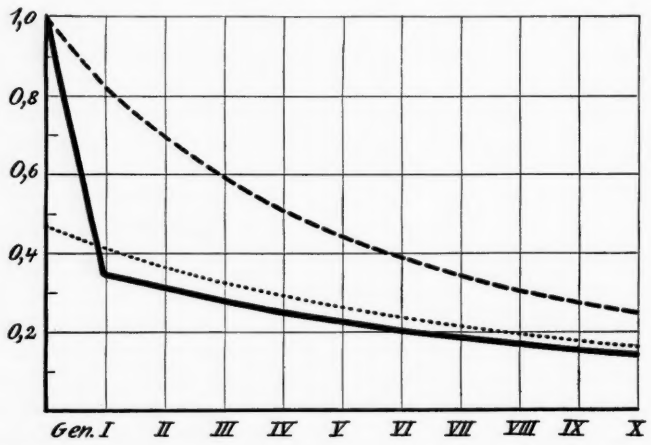

Fig. 29. Monomeric recessivity. Assortative mating, k 0,30. Initial frequency of *RR* 1 per cent. Effect of elimination of *RR* (unbroken line) compared with the effect in panmixia (dashed line).

assortative mating of 30 per cent intensity after equilibrium being reached. This holds true for the initial (random mating) frequency of 0,47 per cent and the gene frequency 6,856 per cent; after 0,30 assortative mating in eight generations the recessive homozygotes are almost exactly 1 per cent and the heterozygotes are decreased in number from 12,77 to 11,73 per cent. In Table 22 and Fig. 29 the effect of complete elimination of the recessive homozygotes in ten generations is shown and compared with the effect in random mating. The curve for assortative mating falls in the first generation very strongly, then more moderately. If, for a comparison with the effect in random mating, we start from the same initial frequency of the character-carriers, 1 per cent, the difference in the effect is rather great; in the third generation, for instance, the reduction of recessive homozygotes obtained through the elimination is increased with 76,5 per cent. This is not surprising since in the assortative mating case the frequency is raised considerably above the level corresponding to the frequency of the genes. We have, therefore, good reasons to compare the effect also with the random mating effect attained at the same gene frequency (6,86 per cent) and a homozygote frequency of 0,47 per cent. The frequency is brought down immediately below that attained in the latter case and the lower level is maintained. The difference, however, is not very great and gradually diminished (Fig. 29); in Gen. X the frequencies are 0,165 and 0,149 per

TABLE 23. *Assortative mating, etc. as Table 22 but k 0,10. RR in per cent (figures for R not given).*

	0	I	II	III	IV	V	VI	VII	VIII	IX	X
Panmixia	1,196	0,972	0,825	0,678	0,579	0,500	0,436	0,384	0,340	0,304	0,273
	1,0	0,826	0,694	0,592	0,510	0,444	0,391	0,346	0,309	0,277	0,251
Assort. mating *k.* 0,10	1,196	0,796	0,670	0,573	0,495	0,432	0,381	0,338	0,302	0,270	0,245

cent, respectively (Table 22), in Gen. XX the curves are almost coincident (0,084, 0,078 per cent).

The strong effect obtained immediately, in the first generation, is easily explained; the surplus of recessive homozygotes (*i. e.* the genes brought to light by the assortative mating) is removed by the first elimination.

An assortative mating by far approaching the intensity assumed above is quite unconceivable in man. Let us assume an intensity of 10 per cent which means that 20 per cent of the matings between recessive homozygotes and others are not realized. If the gene frequency is 10 per cent, the frequency of the recessive homozygotes, as DAHLBERG's table shows, after four generations of assortative mating is increased from 1 to 1,2 per cent (the exact figure, employed in the following calculations, is 1,196). The frequency of the heterozygotes is reduced from 18 to 17,6 per cent. The effect of elimination on the frequency of the recessive character-carriers in ten generations is seen in Table 23 and Fig. 30. The effect is markedly increased in the first generations but the influence of the assortative mating is rather limited; in the third generation, for instance, the reduction of the frequency is increased with about 20 per cent. The reduced frequencies lie but little below those obtained in random mating starting from the same gene frequency (homozygote frequency 1 per cent); after a few generations the figures are almost coincident.

The assortative mating assumed in this example must be said to be very intense. It means that 20,8 per cent of the recessive homozygotes instead of 1 per cent mate among themselves. The frequency of such matings is thus multiplied more than 20 times. In most cases such intense assortative mating ist out of the question. Nevertheless, it is perhaps not impossible that about 20 per cent of the feeble-minded (whose defect, however, is mostly not monomerically recessive) mate among themselves.

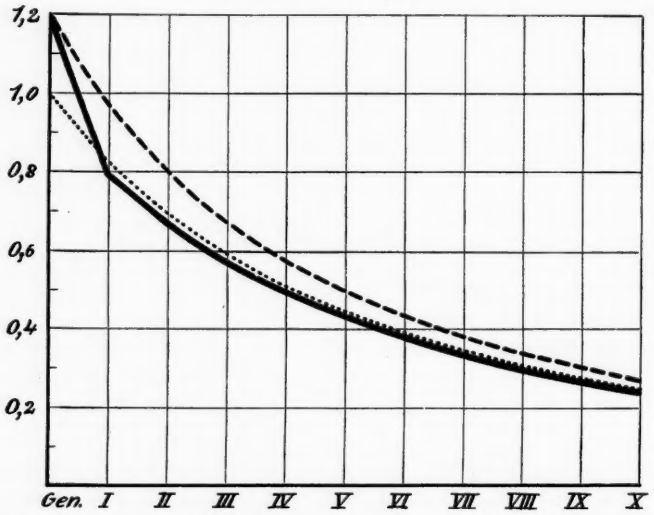

Fig. 30. Monomeric recessivity. Assortative mating, k 0,10. Initial frequency of *RR* 1,2 per cent. Effect of elimination of *RR* compared with the effect in panmixia (dashed line).

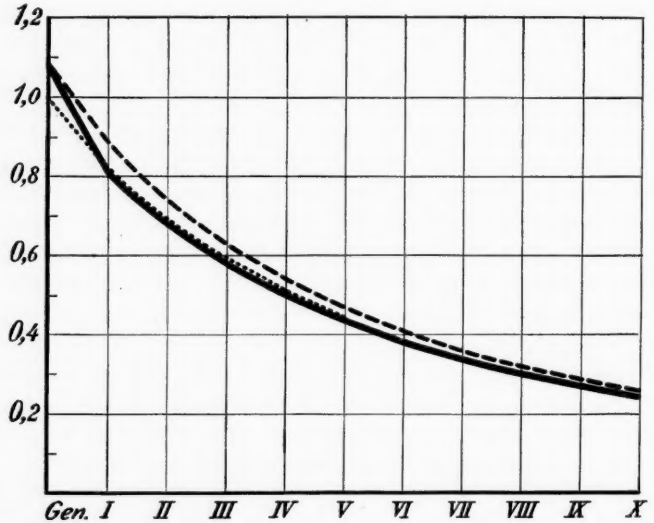

Fig. 31. Monomeric recessivity. Assortative mating, k 0,05. Initial frequency of *RR* 1,089 per cent. Effect of elimination of *RR* compared with the effect in panmixia (dashed line)

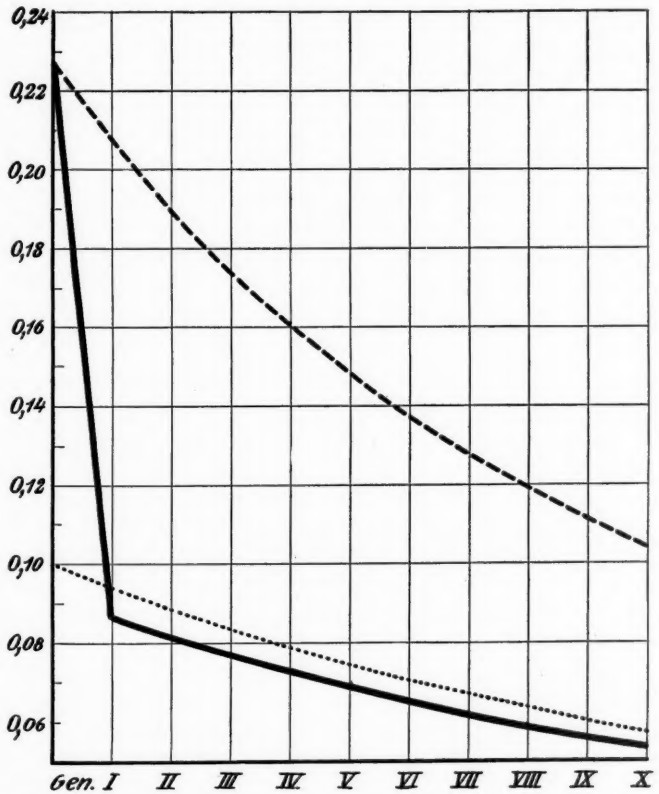

Fig. 32. Monomeric recessivity. Assortative mating, k 0,30. Initial frequency of RR 0,228 per cent. Effect of elimination of RR compared with the effect in panmixia (dashed line).

An assortative mating of 5 per cent (0,05, 10 per cent of the crosses $RR \times DR$ suspended) is rather likely to occur among the feeble-minded. In a case of monomeric inheritance it would mean that 10,9 per cent of the recesssive homozygotes mate with their own kind; the frequency of such mating would be multiplied about eleven times. The effect of elimination is but moderately affected (Fig. 31); in the third generation, for instance, the reduction is 10,7 per cent greater. The difference from the frequencies obtained when starting from the same gene frequency and random mating is quite insignificant; from Gen. V the latter curve could not be continued in the diagram.

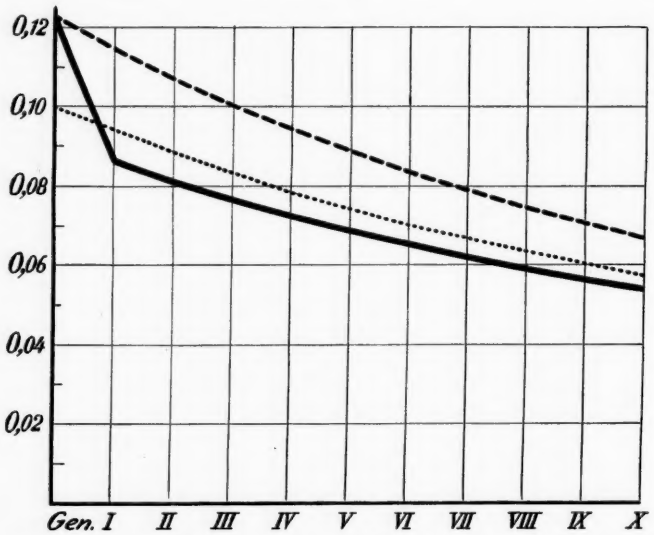

Fig. 33. Monomeric recessivity. Assortative mating, k 0,10. Initial frequency of *RR* 0,123 per cent. Effect of elimination of *RR* compared with the effect in panmixia (dashed line).

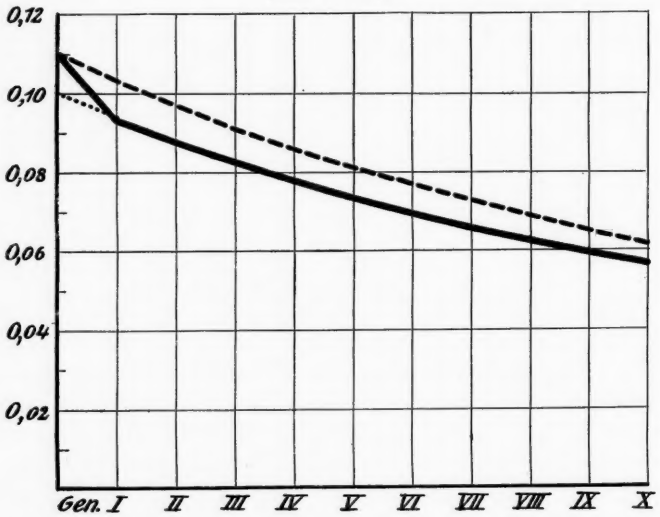

Fig. 34. Monomeric recessivity. Assortative mating, k 0,05. Initial frequency of *RR* 0,11036 per cent. Effect of elimination of *RR* compared with the effect in random mating (dashed line).

The influence of assortative mating is greatly dependent on the frequency of the recessive character and gene: the lower the frequency, the greater is the increase on the effect of elimination of the recessive homozygotes. On the other hand, really rare character-carriers are as a rule not likely to show any considerable assortative mating, at least not in man. There is, however, one exception; the deaf-mutes, as is well known and easily explained, show a marked tendency to mate among themselves. (An investigation on the intermarriage rate of deaf-mutes would be of considerable interest; in panmixia the chances for a hereditary deaf-mute to mate another would be extremely small, about 1 : 5000.) With this exception the following remarks have, as far as man is concerned, a merely theoretical interest.

If a recessive gene has a frequency of $\sqrt{0{,}001}$ (3,162278 per cent) and an assortative mating of 0,30 is assumed, which is of course still more inconceivable than in the higher frequency discussed above, the frequency of the recessive homozygotes will be raised in eight generations from 0,1 to 0,228 per cent (the heterozygotes diminished from 6,13 to 5,87 per cent). Elimination of the recessive homozygotes has, as shown in Fig. 32, immediately a very strong effect. The reduction is, in the third generation, almost twice (182 per cent) as great as in random mating. The curve lies, from the first generation, somewhat below the random mating curve for the same gene frequency.

The corresponding curves for an assortative mating of 0,1 are given in Fig. 33. The effect is still considerable. The frequency of the recessive homozygotes is in four generations increased from 0,1 to 0,12308 per cent. The increase of the reduction is in the third generation 103 per cent.

An assortative mating of 0,05 gives a rather limited effect shown in Fig. 34. The frequency of the recessive homozygotes is in five (four) generations raised to 0,11036 per cent. The effect of elimination of the recessive homozygotes is in Gen. III augmented by 41 per cent but is yet very moderate. The curve is from the first generation practically coinciding with the random mating curve for 0,1 per cent.

We now assume the very low gene frequency of 0,01 (1 per cent) giving in random mating a recessive homozygote frequency of 0,01 per cent (1 in 10.000). An assortative mating of 0,3 would raise in ten generations this frequency to 0,0242 per cent (heterozygotes diminished from 1,98 to 1,95 per cent). Elimination of the recessive homozygotes immediately brings the frequency down very strongly, to 0,00943 per cent, *i. e.* to 39 per cent of the initial frequency (Fig. 35). The curve then lies very little below the random mating curve for 0,01 per cent. The random

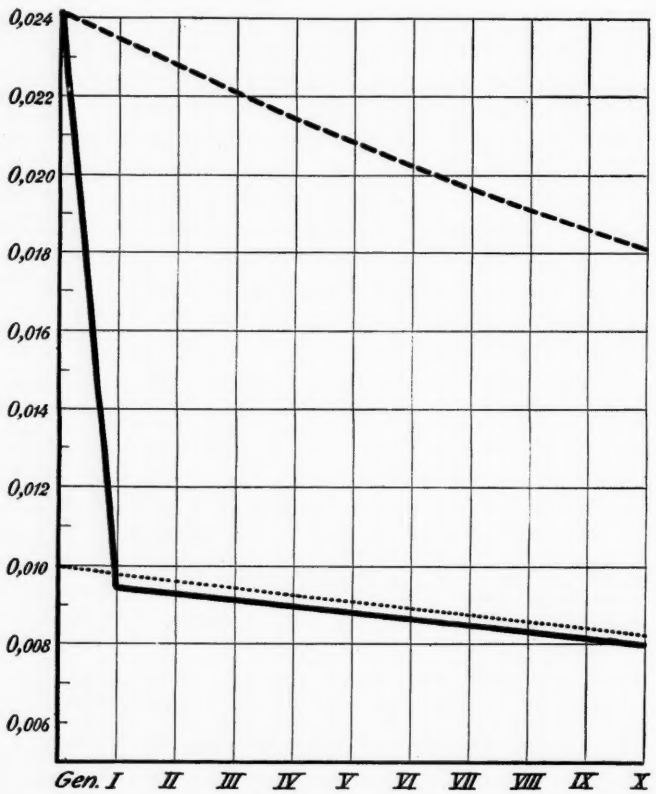

Fig. 35. Monomeric recessivity. Assortative mating, k 0,30. Initial frequency of *RR* 0,024. Effect of elimination of *RR* compared with the effect in random mating (dashed line).

mating effect is in several generations multiplied several times; in Gen. III the reduction is not less than 664 per cent of that obtained in random mating.

We pass directly to an assortative mating of 0,05. The frequency of the recessive homozygotes is in four generations raised to 0,010866 per cent (the reduction of the heterozygotes is quite insignificant, from 1,98 to 1,978 per cent). The effect of elimination is relatively much increased (Fig. 36); in Gen. III the frequency of the recessive homozygotes is reduced more than twice as much (increase of the reduction 126 per cent) as in random mating. Nevertheless, the reduction is but insignific-

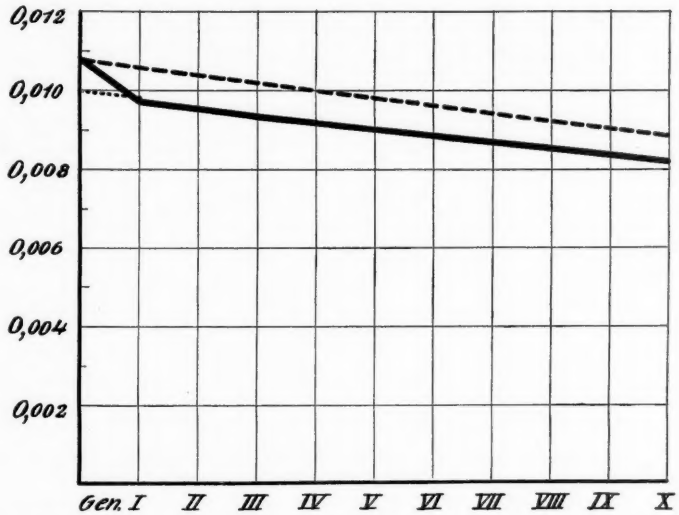

Fig. 36. Monomeric recessivity. Assortative mating, k 0,05. Initial frequency of *RR* 0,01087 per cent. Effect of elimination of *RR* compared with the effect in random mating (dashed line).

ant (in the first generation from 10,9 to 9,8 in 100.000). The curve is from the first generations coincident with the random mating curve for 0,01 per cent.

With the great scarceness of the character-carriers assumed in the last example even an assortative mating of this low intensity cannot, as a rule, occur in man. An exception is, as was pointed out above, hereditary deaf-muteness which, however, is a little more common. It has perhaps some interest to add this example and to consider what would be the effect of elimination of the defectives from reproduction, if a frequency of 0,02 per cent and an assortative mating of 0,05 is assumed. The gene frequency would be 1,34164 per cent giving in panmixia a frequency of 0,018 per cent. The raised frequency 0,02 per cent at once, after one generation, is reduced to 0,01745 instead of 0,01945 per cent if the frequency 0,02 had been a result of panmixia (Fig. 37). From then on, the curve coincides with the random mating curve for 0,018 per cent. The relative increase of the effect of elimination is rather great; the reduction of the frequency of the defect is in Gen. I 464, in Gen. V 162, in Gen. X 129 per cent of the reduction in panmixia. The absolute decrease, however, is still very moderate, in Gen. V, for instance, the

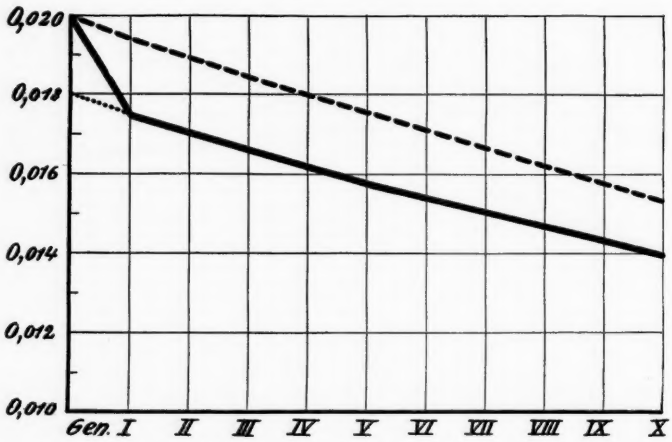

Fig. 37. Monomeric recessivity. As Fig. 36 (k 0,05) but initial frequency of *RR* 0,02 per cent.

reduced frequency is 0,0158 per cent instead of 0,0174 in panmixia; in Gen. X the corresponding figures are 0,014 and 0,01535 per cent.

As a general result of the preceding deductions may be emphasized that the increase of the diminution effect caused by assortative mating, whether great or small, is obtained immediately, in the first generation. The explanation, which is a very simple one, is given above. The frequency is brought down to and even a little lower than the frequency which would result if the assortative mating were suspended. After the first generation the difference in the reduction is gradually diminished; the assortative mating curve and the random mating curve approach and will finally practically (although not mathematically) coincide. In intense assortative mating the distance is still after many generations fairly great. In high frequencies and a low rate of assortative mating (Fig. 31) the curves lie soon very close but they are not very remote from the beginning. In low frequencies and slight assortative mating the distance is from the outset small and diminishes very slowly (if the curves in Fig. 36 are continued, the distance in Gen. L is still more than a third of that in Gen. X).

The strong effect obtained in the first generation explains that from then on the curve of reduction lies more or less close to the random mating curve corresponding to the same gene frequency. In intense assortative mating and fairly high gene frequency the distance between the curves is at first comparatively great (Figs. 29 and 32) as also in

TABLE 24. *Complete assortative mating in dimeric inheritance.*

Gene frequency	Gen. 0 Character-carriers in per cent	Gen. I Character-carriers in per cent	Gen. I Corresponding frequency in monomeric inheritance
2 *A*: 1 *a*:2 *B*: 1 *b*	*aabb* 1,23	2,22	2,22
1 *A*: 9 *a*:9 *B*: 1 *b*	*aabb* 0,81	1,49	1,49
4 *A*: 1 *a*:1 *B*: 4 *b*	*aabb* 2,56	4,41	4,41
1 *A*: 9 *a*:1 *B*: 9 *b*	*A-B-* 3,61	4,97	6,12
1 *A*:19 *a*:1 *B*:19 *b*	*A-B-* 0,95	1,36	1,73
4 *A*: 1 *a*:1 *B*: 4 *b*	*aaB-* 1,44	2,54	2,57
5 *A*: 1 *a*:1 *B*: 5 *b*	*aaB-* 0,849	1,533	1,554
9 *A*: 1 *a*:9 *B*: 1 *b*	*aaB-* 0,990	1,802	1,808
7 *A*: 1 *a*:4 *B*: 4 *b*	*aaB-* 1,172	2,123	2,115
9 *A*: 1 *a*:5 *B*: 5 *b*	*aaB-* 0,750	1,385	1,380
20 *A*:20 *a*:1 *B*:39 *b*	*aaB-* 1,23	1,85	2,22

somewhat lower assortative rate if the frequency is low (Fig. 33). In feeble assortative mating and a more or less low gene frequency the curves are practically coincident (Figs. 34, 36 and 37).

Although in this section only monomeric inheritance is treated, a few words may be added on the influence of assortative mating in cases of dimeric inheritance. It is sufficient to compare, starting from the same initial frequency of the character-carriers, the increase of frequency obtained in monomeric recessivity and in different cases of dimeric inheritance. Such a comparison, for frequencies of about 1 per cent and in two cases for higher frequencies, is given in Table 24. To simplify the matter, complete assortative mating is assumed. Only the increase in the first generation is calculated. For the genes the symbols *A*, *a*, *etc.* are used.

In dimeric recessivity the increase is exactly the same as in monomeric recessivity. In dimeric dominance (both genes more or less rare) the frequency is less increased; in the two examples given it is about 80 per cent of that in monomeric recessivity. In recessivity-dominance the result is variable but the difference is in most cases quite insignificant. When one recessive gene is common and the dominant allele is rare and consequently plays the more important role (the last example in the table), the difference is greater (frequency 83 per cent of that in monomeric recessivity). When the relative frequency of the genes is reverse (examples 2 and 3 from the bottom), the increase is a little greater than in monomeric recessivity but the difference is next to none.

The difference in the increase of the frequency of the character-carriers obviously is not so great as to cause any marked difference in the effect of elimination of the character-carriers, especially when the assortative mating is partial and not too intense.

DIMERIC AND POLYMERIC INHERITANCE.

Hereditary defects with more or less simple and well limited somatic characters are most commonly the effect of a single gene. Mental characters, abnormal as well as normal, which show a great variation cannot possibly have such a simple basis. It is therefore important to analyse the theoretical effect of negative selection of character-carriers in cases of dimerism and polymerism.

The relation between the nature (dominant or recessive) and frequency of the genes and the effect of selection is of course much more complicated than in monomerism. In cases of dimerism, however, calculations are relatively simple.

In dimerism and polymerism the composition of a population mating at random is easily calculated, if genic equilibrium prevails, *i. e.* if the genes are completely mixed. If they are not, in other words if genes are united in the same gametes to a larger extent than what is due to chance, the relative frequency of the zygotes depends not merely on the gene ratio but also on the relative frequency of the gametes, and equilibrium is thus abolished. At random mating it is slowly restored, comparatively rapidly in dimerism, more slowly the higher the number of genes involved. These problems have been treated from a mathematical point of view by WEINBERG (1909), PHILIPTSCHENKO (1924) and particularly in an important investigation by WAHLUND (1928).

In the following the starting point for the calculations and deductions is always a population supposed to be in genic equilibrium. In cases of selection in several subsequent generations regard is paid to the effect of a re-establishment of equilibrium.

Only total selection in autosomal inheritance is treated. I have not paid regard to linkage.

Problems of selection in dimerism and polymerism have been treated by ZIRKLE (1926), who deals with di- and polymeric dominance and di- and polymeric recessivity (not recessivity-dominance). During my calculations and the elaboration of my results I missed seeing his treatise. He gives formulae for calculating in any generation the ratio of the gametes (in dimerism *AB*, *Ab*, *etc.*) from the proportions in the preceding generation (*i. e.* recursion formulae). From these frequencies, the frequencies of the character-carriers are calculated. It must be remarked that (1) what is obtained is the »actual frequency» (*cf.* below), not the frequency resulting in equilibrium (which may, of course, also be calculated), (2) when the zygotes are heterozygous for more than one gene, the result holds only for the first generation (ZIRKLE's F_3); from then on a recombination of genes (»shifting») occurs, which causes rather intricate complications. On that account and because of a somewhat entangled manner of reasoning the practical usefulness of the method is, if I am

not mistaken, considerably reduced. Anyhow, the discussions contain interesting theoretical statements, perhaps more than what is noticed on a cursory perusal of the contents.

Selection in dimerism is also handled by GEPPERT (1938) though in a rather abstract and, as it seems to me, imperspicuous way. He points out that selection leads towards a final state in which one of the genes and, consequently, the character-carriers are removed. Serviceable formulae or graphs are not given. DAHLBERG hardly touches upon this subject (in writing his treatise he knew that I had been working with these problems).

EFFECT OF SELECTION IN ONE GENERATION.

In order to make the subject clear it seems appropriate to consider at first the effect of a single elimination of the character-carriers in one generation, as it appears under the assumption that after selection the genes become completely mixed and thus equilibrium is restored, in other words the state that *would* be realized in the first generation if the genes were mixed and *will* be obtained when after many generations mixing is accomplished and equilibrium established. What is actually obtained in the first generation is another effect, and this effect is always more or less stronger. This is due to the fact that through the elimination the relation between the genotypes is more or less severely disturbed; the character-carriers are, until equilibrium is restored, less in number than what would be expected from the frequency of the genes (see further under the next heading). When one generation only is taken into consideration, the effect due to the frequency of the genes provides a greater interest as, in one way, more truly reflecting the true effect and is more appropriate for comparisons between different cases. We must bear in mind, however, that the frequencies obtained in this manner are minima and that the actual effect on the phenotypes is stronger, sometimes considerably stronger.

DIMERIC RECESSIVITY.

The two pairs of alleles in dimerism should, according to the most common terminology, be called D_1—R_1 and D_2—R_2; the frequencies of the genes are then d_1, r_1, d_2, r_2. To find the frequency of the double recessive homozygotes after total elimination in one generation, the frequency of each gene must be diminished by the frequency of the character-carriers ($r_1^2\, r_2^2$). A formula for the frequency of the recessive homozygotes after selection is rather simple but gives a somewhat bewildering impression, and this is still more the case in recessivity-dominance. I prefer a system of designation which is perhaps a little circum-

locuted but, according to my experience, has the practical advantage of leading to a more rapid calculation. The recessive genes are simply indicated by *a* and *b*, their frequency by the same letters (a and b). For the frequency of the character-carriers a sole letter, u, is used. In the following survey the formula is given in both forms. It should be emphasized once more that the formula gives the genic effect, *i. e.* the effect if equilibrium between the genes is assumed.

Dimeric recessivity.

Elimination of $R_1R_1R_2R_2$ (*aabb*).
Recessive genes R_1, frequency r_1,
R_2, » r_2.

$$r_1^2 r_2^2{}_1 = \frac{(r_1 - r_1^2 r_2^2)^2 (r_2 - r_1^2 r_2^2)^2}{(1 - r_1^2 r_2^2)^4}.$$

or

Recessive genes *a*, frequency a.
b, » b.
Character-carriers, frequency $a^2b^2 = u$.

$$u_1 = \frac{(a - u)^2 (b - u)^2}{(1 - u)^4}.$$

Table 25 gives examples of the effect of elimination in one generation of dimeric recessive homozygotes as compared with the effect in monomeric recessivity, *i. e.* the effect which would be obtained if the character were determined by one sole recessive gene. Only frequencies from 6,25 per cent downwards are given. The examples are not chosen very methodically; to a certain extent I have added cases in which I have once for some reason made calculations.

A glance at the table shows that, on the whole, the same general rules prevail as in monomeric recessivity: in high frequencies of the recessive homozygotes their elimination causes a strong reduction; in low frequencies the effect is feeble, in very low frequencies practically none. The reduction, however, does not sink regularly with decreasing frequency; there are considerable irregularities, due to the fact that the frequency of both genes and their dominant alleles influences the result. Five types of relations between the genes may be discerned (in the table separated by horizontal lines). What is said below bears primarily on the frequencies of the table.

I. The two recessive genes have the same frequency. If both have

TABLE 25. *Dimeric recessivity. Elimination of double recessive homozygotes aabb in one generation. In the calculations more decimals are employed as shown in the table.*

Gene ratio				Gen. 0	Gen. I		Gen. I in monomeric recessivity		Reduction in per cent of the reduction in monomeric recessivity
A	*a*	*B*	*b*	Frequency	Frequency	In per cent	Frequency	In per cent	
1	1	1	1	6,25	4,74	75,9	4,00	64,0	67,0
3	2	3	2	2,56	2,18	85,1	1,90	74,3	57,8
2	1	2	1	1,23	1,12	88,2	1,00	81,0	50,7
4	1	4	1	0,160	0,156	97,5	0,148	92,3	33,3
5	1	5	1	0,077	0,076	98,5	0,073	94,7	28.9
9	1	9	1	0,010	0,009864	99,6	0,009803	98,0	18,3
3	2	2	3	5,76	4,37	75,9	3,75	65,0	68,9
2	1	1	2	4,94	3,76	76,2	3,31	66,9	72,0
3	1	1	3	3,52	2,72	77,4	2,49	70,9	77,7
4	1	1	4	2,56	2,02	79,0	1,90	74,3	81,6
5	1	1	5	1,93	1,56	80,6	1,49	77,1	84,7
9	1	1	9	0,810	0,694	85,7	0,682	84,2	90,4
19	1	1	19	0,226	0,207	91,6	0,206	91,1	95,2
99	1	1	99	0,0098	0,009612	98,1	0,009610	98,05	98,9
12	8	11	9	3,24	2,69	82,9	2,40	82,9	65,7
7	3	6	4	1,44	1,26	89,2	1,48	89,2	53,0
4	1	3	2	0,640	0,596	93,1	0,549	85,7	48,4
6	1	4	3	0,375	0,355	94,6	0,333	88,8	48,3
5	1	4	2	0,309	0,295	95,7	0,277	89,7	41.7
6	1	5	2	0,167	0,162	97,3	0,154	92,3	36,3
9	1	6	4	0,160	0,155	96,6	0,148	92,5	44,1
7	1	6	2	0,0977	0,0958	98,1	0,0918	95,4	32,5
14	1	11	4	0,0316	0,1313	98,9	0,0305	96,6	30,6
15	5	1	19	5,64	3,77	66,9	3,68	65,3	95,3
80	20	1	99	3,92	2,74	70,0	2,73	69,7	99,1
16	4	1	19	3,61	2,60	72,0	2,55	70,6	95,2
8	2	1	9	3,24	2,41	74,4	2,40	74,0	98,4
17	3	1	19	2,03	1,58	77,7	1,56	76,6	95,0
4	1	2	3	1,44	1,25	86,9	1,48	79,7	64,4
5	1	2	4	1,23	1,07	86,3	1,00	81,0	69,5
90	10	1	99	0,980	0,813	83,0	0,811	82,8	99,0
18	2	1	19	0,902	0,760	84,3	0,752	83,4	99,9
9	1	2	8	0,640	0,566	89,3	0,549	85,7	80,9
9	1	4	6	0,360	0,332	92,3	0,320	89,0	70,2
19	1	2	18	0,203	0,187	92,4	0,185	91,6	90,4

Gene ratio				Gen. 0	Gen. I		Gen. I in monomeric recessivity		Reduction in per cent of the reduction in monomeric recessivity
A	*a*	*B*	*b*	Frequency	Frequency	In per cent	Frequency	In per cent	
4	2	3	3	2,78	2,33	83,9	2,04	73,5	60,7
3	1	2	2	1,56	1,37	87,8	1,23	79,0	57,9
5	1	3	3	0,694	0,638	91,8	0,592	85,2	55,2
9	1	5	5	0,250	0,238	95,1	0,227	90,7	37,1
19	1	10	10	0,0625	0,0617	98,7	0,0595	95,2	26,1

the frequency 0,5 (gene ratio 1 : 1 : 1 : 1), the reduction is 67 per cent of the reduction in monomeric recessivity. This percentage *sinks* with diminishing frequency of the recessive genes and homozygotes. In the initial frequency 0,01 per cent it is only 18,3.

II. One of the recessive genes (*a* in the table) has a lower frequency. Distribution of the genes »symmetrically», *i. e. a* has the frequency of *B*, *b* that of *A* (3 : 2 : 2 : 3, 2 : 1 : 1 : 2, *etc.*). In the highest frequencies of the table the relative reduction (in per cent of the reduction in monomeric recessivity) is slightly feebler than in the highest frequency in the former type (1 : 1 : 1 : 1). The percentage *rises* with sinking frequency, which implies a reduced frequency of the one and an increased frequency of the other gene; in the last example of the table (*a* initially 1, *b* 99 per cent) the effect is almost the same as in monomeric recessivity; the effect being determined of course almost exclusively by the rare gene.

III. One of the recessive genes (*a*) has a lower frequency. Distribution of the genes »asymmetrically». In both pairs of alleles the dominant gene has a higher frequency than the recessive one; the genic distribution therefore tends towards type I. In distributions approaching the ratio 1 : 1 : 1 : 1 the relative reduction is slightly feebler than in this relation. The percentage *sinks* with diminishing frequency of the recessive homozygotes and increasing predominance of the dominant allele of one of the recessive genes; but increasing predominance of the dominant allele of the other pair has an opposite effect of considerable magnitude.

IV. One of the recessive genes (*a*) has a lower frequency. Distribution of the genes asymmetrically. In one pair the dominant gene and in the other the recessive gene has a higher frequency; the genic distribution therefore tends towards type II. In frequencies approaching

1 : 1 : 1 : 1 the relative reduction is slightly feebler than in this distribution. It *rises* (towards 100 per cent) with sinking frequency of the recessive homozygotes, but the increase is worked against by increasing frequency of the dominant gene of the other pair.

V. One of the recessive genes (*a*) has a lower frequency. Distribution of the genes asymmetrically. The more common recessive gene and its dominant allele have the same frequency (0,5). The distribution of the genes is thereby intermediate between III and IV and tends neither towards I nor towards II. The relative reduction lies in the highest frequencies a little below the reduction in 1 : 1 : 1 : 1 and *sinks* towards 25 per cent.

The distribution of the genes in the population is easily calculated (panmixia presumed). Three examples may be of some interest. In the gene ratio 2 : 1 : 2 : 1, where the reduction is only half that of the reduction in monomeric recessivity, one or both genes are present in about 80 per cent of the population. In the ratio 9 : 1 : 1 : 9 *a* or *b* or both are present in somewhat more than 99 per cent, in the ratio 90 : 10 : 1 : 99 in 99,99 per cent, *i. e.* practically in all individuals. And yet elimination of the character-carriers has in the former case almost the same, in the latter practically quite the same effect as in monomeric recessivity. This may appear paradoxical but is in reality very simple and natural.

DIMERIC RECESSIVITY-DOMINANCE.

If a character showing dimeric inheritance is due to one recessive and one dominant gene and only the dominant homozygotes (*aaBB*) are eliminated, the result is the same as in dimeric recessivity. The formula for the reduction is the same, if *b* is substituted by *B*. This case has but a limited interest.

An important problem is, on the other hand, the effect of elimination of the character-carriers in cases of complete dominance of the dominant gene. A formula for the reduced frequency after selection in one generation is given below:

Dimeric recessivity-dominance.

Elimination of $aaBB + aaBb$
Genes *a*, frequency a.
B, » B.
b, » b.
Character-carriers *aaBB*, frequency $a^2B^2 = v$.
aaBb, » $2a^2Bb = w$.

$$(v+w)_I = \frac{(a-(v+w))^2\left(B-\left(v+\frac{w}{2}\right)\right)^2+2(a-(v+w))^2\left(B-\left(v+\frac{w}{2}\right)\right)\left(b-\frac{w}{2}\right)}{(1-(v+w))^4}.$$

Examples of the reduction obtained are given in Table 26. They are not more systematically chosen than in Table 25. As in this table a new equilibrium between the genes is presumed. It is seen at once and easily understood that there is no regular decrease of the effect with sinking frequency, though the reduction is always more or less strong in high frequencies and small in very low. The relative frequency of the two co-operating genes (*a* and *B*) plays, of course, an important role: the rarer the dominant gene, the stronger is the effect of the selection. In very low frequencies of this gene, approaching cases of monomeric dominance (the recessive gene present in all individuals), the dominant gene is immediately almost totally removed. On the other hand, when the dominant gene is more common than the recessive one, the effect approaches the effect in monomeric recessivity. The frequency of the two other alleles (*A* and *b*) has, however, also a great importance. Five types of relations between the genes may be discerned.

I. The recessive and the dominant gene have the same frequency (2 : 1 : 1 : 2, *etc.*). In high frequencies the reduction is not much less than in monomeric recessivity (in the highest frequency of the table 91,5 per cent). The relative reduction *sinks* with diminishing frequency. In very low frequencies the reduction is less than half of the reduction in monomeric recessivity but is quite insignificant in both cases.

II. The dominant gene *B* has a lower frequency than *a*, whose frequency is lower than that of *A*. The relative reduction *rises* (in the table from 73 per cent) with sinking frequency of *B* (approaching the reduction in monomeric dominance) but the frequency of *a* also affects the result: the higher it is, *i. e.* the lower the frequency of *A*, the stronger is the effect in relation to the reduction in monomeric recessivity.

III. *B* has a lower frequency than *a* which has the same frequency as *A*. The relative reduction is always high and *rises* with sinking frequency of *B*. Already from a frequency of about 2,5 per cent the reduction is *stronger* than in monomeric recessivity.

IV. *B* has a lower frequency than *a*, whose frequency is higher than that of *A*. The relative reduction is strong. It *rises* with sinking frequency of *B* and rising frequency of *a*.

V. *B* has a higher frequency than *a*, whose frequency is lower than that of *A*. The relative reduction is high but not higher than in mono-

TABLE 26. *Dimeric recessivity-dominance. Elimination of dominant character-carriers aaBB + aaBb (aaB-, cf. below) in one generation. In the calculations, more decimals were employed than given in the table.*

Gene ratio				Gen. 0	Gen. I		Gen. I in monomeric recessivity		Reduction in per cent of the reduction in monomeric recessivity
A	a	B	b	Frequency	Frequency	In per cent	Frequency	In per cent	
2	1	1	2	6,17	4,45	72.2	3,96	64,2	91,5
7	3	3	7	4,59	3,48	75,9	3,11	67,8	75,0
4	1	1	4	1,44	1,25	86,6	1,15	79,7	66,1
6	1	1	6	0,541	0,500	92,4	0,469	86.8	57,3
9	1	1	9	0,190	0,182	95,5	0,174	91,8	50,1
19	1	1	19	0,0244	0,0241	98,9	0,0236	96,9	37,7
3	2	1	4	5,76	4,29	74,4	3,75	65,0	73,2
4	2	1	5	3,40	2,72	80,1	2,42	71,3	69,3
6	4	1	9	3,04	2,41	79,3	2,20	72,5	75,6
7	3	1	9	1,71	1,46	85,6	1,34	78,2	66,6
12	8	1	19	1,60	1,27	79,6	1,26	78,8	96,3
18	12	1	29	1,05	0,865	82,4	0,863	82,3	99,2
8	2	1	9	0,760	0,692	91,1	0,643	84,6	58,1
5	5	1	9	4,75	3,41	71,9	3,20	67,4	86,3
10	10	1	19	2,44	1,79	73,5	1,82	74,8	105,2
20	20	1	39	1,23	0,917	74,3	1,0	81,0	135,4
25	25	1	49	0,990	0,740	74,7	0,819	82,7	99,5
50	50	1	99	0,497	0,335	67,3	0,434	87,3	256,2
1	19	1	19	8,880	0,954	11,2	5,27	59,3	219,8
4	6	1	9	6,84	4,32	63,2	4,30	62,8	99,1
5	15	1	19	5,48	2,48	44,3	3,60	65,7	159,8
1	49	1	49	3,80	0,133	3,6	2,66	70,0	321,4
1	99	1	99	1,95	0,040	2,0	1,50	77,0	448,1
30	70	1	99	0,975	0,062	6,4	0,808	82,8	545,4
4	1	4	1	3,84	2,71	75,7	2,68	69,9	98,7
4	1	3	2	3,36	2,48	73,8	2,40	71,4	91,5
4	1	2	3	2,56	2,03	79,2	1,90	74,3	81,0
5	1	4	2	2,47	1,88	76,2	1,84	74,7	94,2
5	1	3	3	2,08	1,66	79,5	1,59	76,4	86,8
6	1	6	1	2,00	1,54	77,0	1,53	76,8	98,9
8	1	8	1	1,22	0,990	81,2	0,988	81,0	99,0
9	1	9	1	0,990	0,820	82,8	0,819	82,7	99,5
9	1	7	3	0,910	0,765	77,3	0,759	76,7	95,7
9	1	5	5	0,750	0,650	86,7	0,635	84,7	86,8
14	1	9	6	0,373	0,335	89,7	0,330	88,3	88,1
49	1	49	1	0,040	0,0384	96,1	0,0384	96,1	99,95
99	1	99	1	0,010	0,0098	98,0	0,0098	98,0	99,95

meric recessivity. It *rises* with rising frequency of *B* and sinking frequency of *a*.

The distribution of the genes in the population varies for obvious reasons (one of the genes dominant) very strongly. In the extreme case where *a* is very common and *B* very rare almost all individuals possess one of the genes or both (99,99 per cent in the gene ratio 1 : 99 : 1 : 99). In the opposite extreme case where *B* is very common and *a* very rare one of the genes or both are present only in a small fraction of the population (in the ratio 99 : 1 : 99 : 1 in 1,99 per cent). In type III the genes or one of them are always present in 75 per cent of the population. In most cases the percentage lies between 75 and a little less than 20 per cent.

DIMERIC DOMINANCE.

Table 27 gives some examples of elimination of double dominant character-carriers. It is assumed, in other words, that a character is caused by two co-operating dominant genes *A* and *B* and that all individuals possessing both these genes (*AABB*, *AABb*, *AaBB* and *AaBb*) are eliminated. — It seems desirable, on practical grounds, to have a common designation covering both homozygous and heterozygous character-carriers. In the following, a hyphen (-) after a single symbol for a dominant gene represents its dominant *or* its recessive allele. *AAB-* thus means *AABB* and *AABb*, *etc.*

When one of the two dominant genes is rare and the other common, the conditions approach those prevailing in monomeric dominance, and the reduction is very strong, stronger the greater the difference in frequency is. In the more or less low frequencies of the table the character and gene almost disappear after selection in one generation. When both dominant genes are rare and have the same frequency, the reduction is feebler than in monomeric recessivity but in the frequencies of the table not much feebler. In very low frequencies the reduction is practically the same as in monomeric recessivity. Between these two cases there is a complete series of intermediate cases. When one of the dominant genes is rare and the other has the same frequency as its recessive allele, the reduction is much stronger than in monomeric recessivity, and it is stronger the rarer the rare gene. To sum up: The effect of selection is in low frequencies of both genes about the same as in monomeric recessivity or not much less. It rises with rising frequency of one of the genes, tending to a reduction several hundred times of that in monomeric recessivity.

TABLE 27. *Dimeric dominance. Elimination of character-carriers AABB, AABb, AaBB and AaBb = A-B-. In the calculations more decimal places are employed than shown in the table.*

Gene ratio				Gen. 0	Gen. I		Gen. I in monomeric recessivity		Reduction in per cent of the reduction in monomeric recessivity
A	*a*	*B*	*b*	Frequency	Frequency	In per cent	Frequency	In per cent	
1	49	49	1	3,96	0,0017	0,042	2,75	69,6	328,4
1	99	99	1	1,99	0,0002	0,010	1,53	76,8	431,0
1	99	50	50	1,49	0,379	25,4	1,19	79,4	363,4
1	19	1	19	0,951	0,811	85,3	0,789	83,0	86,4
1	199	100	100	0,748	0,203	27,1	0,634	84,7	476,9
1	99	10	90	0,378	0,303	80,1	0,336	88,7	176,7
1	199	40	160	0,359	0,146	40,7	0,311	86,6	440,3
1	49	2	48	0,310	0,277	89,3	0,279	89,7	105,1
1	39	1	39	0,244	0,222	90,9	0,221	90,8	98,8
1	99	5	95	0,194	0,166	85,7	0,178	91,7	173,0
1	99	1	99	0,0396	0,0381	96,2	0,0381	96,1	99,6

The distribution of the genes in the population is, except extreme cases where one of them has a very great predominance, less than in double recessivity and recessivity-dominance. In the gene ratio 1 : 19 : 1 : 19, for instance, *A* and *B* or both are present in 18,5 per cent of the individuals. In the ratio 1 : 99 : 10 : 90 the corresponding percentage is 20,6, in 1 : 99 : 1 : 99 it is 3,94.

TRIMERISM.

When a character is due to three co-operating genes, elimination of the character-carriers acts principally as in dimerism but the variations are very great owing to the great number of possible combinations. Some examples are given in Table 28. An analysis is hardly necessary and would in any case require a broader basis than the examples of the table, which are chosen rather at random.

In trimeric recessivity the reduction is always feebler than in corresponding frequencies in monomeric recessivity. When one of the three recessive genes is rare and the two others common, the result is of course determined chiefly by the rare gene and the effect of selection lies more or less near the effect in monomeric recessivity, nearer the greater the difference in frequency is. On the other hand, when the three recessive genes have a markedly lower frequency than the domin-

TABLE 28. *Trimeric inheritance. Elimination of character-carriers in one generation. In the calculations more decimal places are employed than given in the table.*

Gene ratio						Gen. 0	Gen. I		Gen. I in monomeric recessivity		Reduction in per cent of the reduction in monomeric recessivity
A	*a*	*B*	*b*	*C*	*c*	Frequency	Frequency	In per cent	Frequency	In per cent	
								aabbcc			
2	2	2	2	1	3	3,52	2,96	84,1	2,50	71,0	54,9
1	1	1	1	1	1	1,56	1,42	90,8	1,23	87,0	43,6
3	1	1	3	1	3	1,54	1,36	88,3	1,22	78,6	56,2
3	1	1	3	2	2	0,879	0,817	93,0	0,735	83,6	42,7
9	1	1	9	1	9	0,656	0,579	82,2	0,561	85,6	91,4
2	1	2	1	1	2	0,549	0,522	95,1	0,476	86,7	36,6
3	1	2	2	2	2	0,391	0,373	95,4	0,346	89,4	40,0
5	1	3	3	3	3	0,174	0,169	97,6	0,160	92,2	30,8
2	1	2	1	2	1	0,137	0,135	98,4	0,128	93,0	22,8
								aabbC-			
1	1	1	1	1	1	4,69	3,79	80,8	3,17	67,6	59,2
2	2	2	2	1	3	2,73	2,36	86,4	2,01	73,6	51,4
1	100	1	100	1	100	1,89	0,076	4,0	1,46	77,3	264,3
1	5	5	1	5	1	1,87	1,52	81,2	1,45	77,4	83,2
3	1	2	2	2	2	1,17	1,06	90,6	0,954	81,4	50,7
2	1	2	1	2	1	1,10	1,00	91,4	0,899	81,9	47,8
1	9	9	1	9	1	0,802	0,688	85,8	0,675	84,2	94,3
								aaB-C-			
10	10	1	19	19	1	2,43	1,79	73,6	1,82	74,8	105,0
3	1	1	3	2	2	2,05	1,75	85,4	1,57	76,5	60,9
4	1	1	4	4	1	1,38	1,20	87,1	1,11	80,1	64,7
3	1	1	3	1	3	1,20	1,08	90,4	0,972	81,3	51,2
25	25	1	49	49	1	0,990	0,737	74,4	0,819	82,7	148,0
9	1	9	1	9	1	0,880	0,813	83,0	0,811	82,8	99,0
50,5	50,5	1	100	100	1	0,493	0,365	74,1	0,430	87,3	204,6
								A-B-C-			
1	100	100	1	100	1	1,97	0,0004	0,02	1,51	76,9	435,0
1	6	1	6	1	6	1,87	1,61	86,1	1,45	77,4	61,8
1	100	100	1	50,5	50,5	1,48	0,379	0,26	1,82	80,0	372,3
1	19	19	1	1	19	0,948	0,791	83,5	0,787	83,0	97,4
1	19	1	19	10	10	0,713	0,622	87,2	0,606	85,0	85,6
1	9	1	9	1	9	0,686	0,630	92,7	0,585	85,3	55,4
1	19	1	19	5	15	0,415	0,363	87,5	0,367	88,3	108,3
1	100	100	1	1	100	0,0388	0,0373	96,2	0,0373	96,2	99,5

ant alleles and, consequently, the character-carriers are rare, the reduction is insignificant and much less than in monomeric recessivity. Other combinations give transitions between these extremes.

When a rare dominant gene plays an important role, the effect is very strong. The first and third examples of *A-B-C-* and the third example of *aabbC-* are quite extreme and close to monomeric dominance. In the case of three relatively rare dominant genes, of one recessive and two dominant genes having the same frequency, and in cases of two recessive and one dominant gene in which the dominant gene (*C*) has a higher frequency than the two recessive ones or the same frequency as one of them, the reduction is about half of the reduction in monomeric recessivity (also in the second example of *aabbC-*).

SELECTION IN SEVERAL SUBSEQUENT GENERATIONS.

An elimination of character-carriers which is repeated in several subsequent generations has the same general effect in dimeric and polymeric as in monomeric inheritance: the frequency, if not very low from the beginning, sinks at first comparatively strongly, then less and less. In Table 29 some examples are given of the result in dimerism for the three first generations, three examples for dimeric recessivity, four for recessivity-dominance, and one for dimeric dominance. The figures under the heading »Genes completely mixed» indicate the effect which would occur if the genes were completely mixed in each generation. Such a mixing-process would, however, take numerous generations, and this assumption is, therefore, quite unnatural. The figures have, however, a certain theoretical interest. The figures in brackets indicate the corresponding effect in monomeric recessivity.

If we regard the figures showing the actual effect in the third generation, it appears that in certain cases they are markedly higher than those for monomeric recessivity. The difference is, however, not very considerable. In most cases the figures are higher but the difference is more or less insignificant or almost non-existent. In all these cases the results correspond roughly to the effect after elimination in one generation as discussed above. In some cases — where a dominant gene plays an important role — the elimination has a much stronger effect than in monomeric recessivity.

These remarks touch merely upon the surface of the laws regulating the diminution of the character-carriers where elimination is continued in several subsequent generations. The figures in Table 29 are given, mainly because they were calculated in an early stage when I

TABLE 29. *Dimeric inheritance. Elimination in three generations.*

Gene ratio $A : a : B : b$	Frequency of character-carriers in per cent						
	Gen. 0	Gen. I		Gen. II		Gen. III	
		Genes completely mixed	Actual result	Genes completely mixed	Actual result	Genes completely mixed	Actual result
		aabb					
1 1 1 1	6,25	4,74	4,00 (4,00)	3,75	3,10 (2,79)	3,06	2,58 (2,04)
1 5 5 1	1,93	1,55	1,49 (1,49)	1,28	1,21 (1,18)	1,07	1,02 (0,96)
1 9 9 1	0,810	0,694	0,682 (0,682)	0,601	0,591 (0,582)	0,527	0,517 (0,502)
		aaB-					
2 1 1 2	6,17	4,46	3,95 (3,96)	3,37	2,96 (2,75)	2,65	2,39 (2,03)
4 1 1 4	1,44	1,25	1,12 (1,15)	1,08	0,95 (0,94)	0,94	0,84 (0,78)
20 20 1 39	1,23	0,916	0,621 (1,00)	0,688	0,464 (0,826)	0,519	0,387 (0,693)
8 1 8 1	1,20	0,990	0,990 (0,978)	0,819	0,820 (0,810)	0,697	0,690 (0,682)
		A-B-					
1 19 1 19	0,951	0,793	0,415 (0,790)	0,671	0,381 (0,666)	0,575	0,351 (0,569)

had planned a still more superficial treatment of the problems than that presented in this paper. A thorough analysis would involve problems of a most intricate nature having no bearing on the purpose of the present investigation. I have, however, made several calculations and shall give a short exposition of the matter elucidated by a few striking examples. — »Mixing curve» in the following signifies the curve originating under the (unnatural) assumption that the genes are completely mixed after each elimination (*cf.* above p. 235), »actual curve» means the curve resulting through combination, after each elimination of the character-carriers, of the gametes of the remaining individuals.

DIMERIC RECESSIVITY.

The simplest case is dimeric recessivity with equal frequency of the four genes ($1\,A : 1\,a : 1\,B : 1\,b$) and elimination of *aabb* (Fig. 38). The

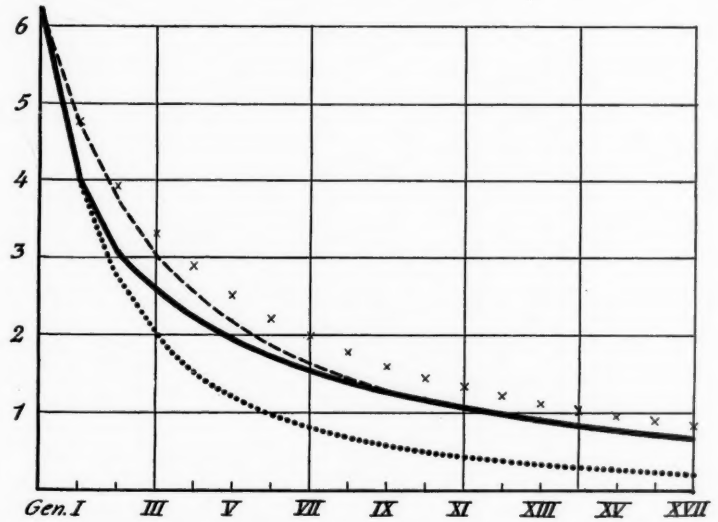

Fig. 38. Dimeric recessivity. Gene ratio 1 *A* : 1 *a* : 1 *B* : 1 *b*. Elimination of *aabb* (initial frequency 6,25 per cent). Unbroken line, actual curve. Dashed line, mixing curve. Dotted line, the corresponding curve for monomeric recessivity. The crosses indicate the frequency after cessation of the selection and equilibrium reached; *cf.* the text.

frequency of *aabb* is reduced much more in the first generation than if mixture of the genes after the elimination is assumed, *viz.* from 6,25 to 4,0 instead of 4,74 per cent (*cf.* Table 29) or exactly as much as in monomeric recessivity (in both cases the same percentage of the gametes, *ab* and *a* respectively, is eliminated). In the following generations the actual curve and the mixing curve converge. They meet in Gen. IX and then diverge but so slightly that they are almost parallel and practically coincident (in the diagram the actual curve only is drawn after Gen. IX). The actual curve and the curve for monomeric recessivity are at first (from Gen. II) divergent but from Gen. VII slightly convergent. The difference between the actual effect and the effect in monomeric recessivity is (with exception of Gen. I) rather great; the diminution obtained, in the latter case, in Gen. IV will be obtained in Gen. VII, the diminution obtained in Gen. VI will be reached in Gen. XII, *etc.* The diminution is in Gen. IV 85,7 per cent of that obtained in monomeric recessivity and the difference slowly decreases (Gen. VII 86,3, Gen. X 88,3 per cent).

Thus, the frequency of the character-carriers (*aabb*) is, in the first generations, markedly more reduced than in the (fictitious) case of a

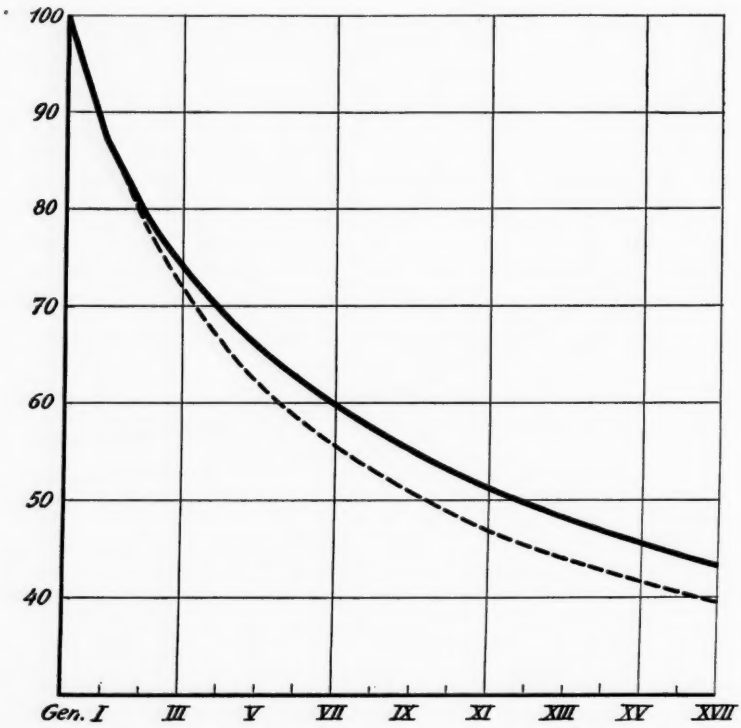

Fig. 39. Dimeric recessivity. Gene ratio $1A:1a:1B:1b$. Frequency of the genes (a and b, respectively). Ordinate, frequency of a in per cent of A (and b in per cent of B). Unbroken line, actual curve; dashed line, mixing curve.

complete mixing of the genes in each generation. This strong diminution is, in one way, only apparent. If the frequency of the genes be regarded instead of that of the individuals, it appears that from the second generation their frequency is less reduced than if mixing be assumed. Fig. 39 illustrates the difference. The actual curve and the mixing curve for the frequency of the genes are at first divergent, then (from Gen. VI to Gen. X) parallel, then very slightly converging. There is, of course, no inconsistency in the result as far as it concerns the character-carriers and the genes. The character-carriers, as far as the genes are not completely mixed, are underrepresented, *i. e.* have a lower frequency than that due to the frequency of the two recessive genes, because the latter to a corresponding extent are still joined to the dominant genes to which

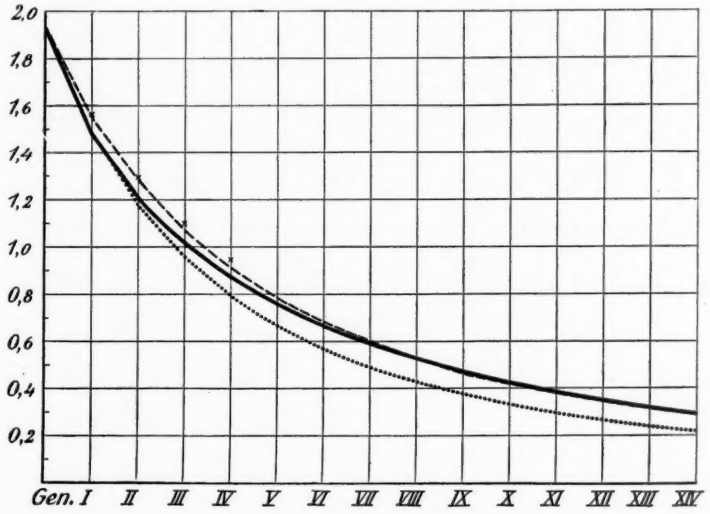

Fig. 40. Dimeric recessivity. Gene ratio 1 *A* : 5 *a* : 5 *B* : 1 *b*. Elimination of *aabb* (initial frequency 1,93 per cent). Signification of the curves and crosses, see Fig. 38.

they were originally united (the gamete ratio after the first elimination is 60 *AB* : 60 *Ab* : 60 *aB* : 45 *ab* = 26,67 *AB* : 26,67 *Ab* : 26,67 *aB* : 20,0 *ab* instead of 64 *AB* : 56 *Ab* : 56 *aB* : 49 *ab* = 28,44 *AB* : 24,89 *Ab* : 24,89 *aB* : 21,78 *ab*). Or, if we start from the genes: the reduction of their frequency in the first generation is the same whether merely a combination of the gametes or a mixing of the genes is assumed (Fig. 39); the frequency of the character-carriers *aabb* is more reduced in the former case because a greater part of the recessive genes are joined to the dominant genes (*a* to *B*, *b* to *A*).

The disturbed relation between the gametes (underrepresentation of *ab* and, consequently, of *AB*) has another interesting consequence. If elimination ceases and equilibrium is slowly approached, the frequency of *aabb*, reduced by the preceding elimination, will slowly rise; when after many generations equilibrium is at last reached, the frequency will lie above that of the mixing curve. In Fig. 38 these frequencies are marked in each generation by a cross. The increase in the first generations lies between 18,5 and 28,7 per cent.

In a practical sense the reduction of the character-carriers must be considered more important than the reduction of the genes. Firstly, in the case of man our interest is centered in what will occur in the nearest

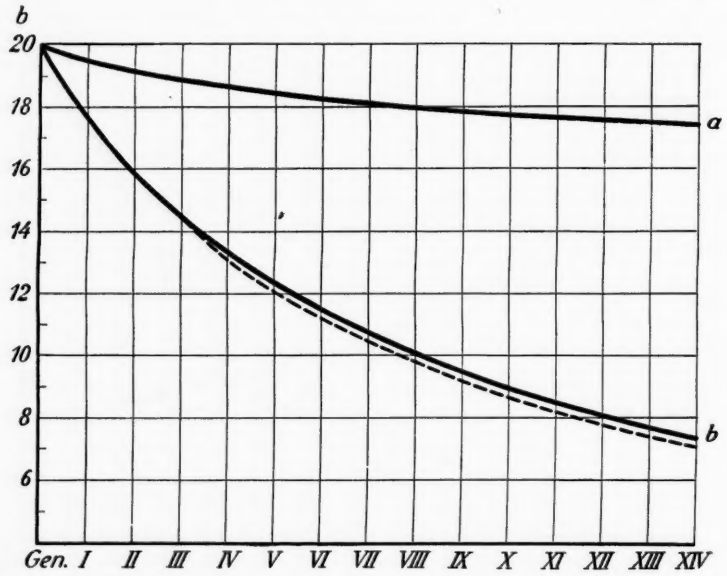

Fig. 41. Dimeric recessivity. Gene ratio 1 *A* : 5 *a* : 5 *B* : 1 *b*. Elimination of *aabb*. Frequency of the genes. Ordinate, frequency of *b* in per cent of *B*. Initial frequency of *a* reduced to the value of *b* (20). Dashed line, mixing curve for *b*.

coming generations. Secondly, equilibrium after cessation of the selection will be reached exceedingly slowly. Finally, as may be seen from the following examples, those frequencies which are of practical interest in the case of man exhibit a very slight increase.

In other cases of dimeric recessivity the result is principally equal. It is illustrated for the gene ratio 1 *A* : 5 *a* : 5 *B* : 1 *b* (initial frequency of *aabb* 1,93 per cent) in Fig. 40, for the ratio 1 *A* : 9 *a* : 9 *B* : 1 *b* (initial frequency of *aabb* 0,81 per cent) in Fig. 42. These cases are interesting because, especially in the latter one, the frequencies approach a value which could be of practical importance in man. Two important points should be noticed. The difference between the actual curve and the mixing curve is but slight and is smaller the more one of the recessive genes prevails. Further, the difference in relation to the effect in monomeric recessivity is rather unimportant, especially in the first generations. In the gene ratio 1 : 5 : 5 : 1 the frequency of *aabb* is, for instance, in Gen. III reduced from 2,0 per cent to 1,0 instead of 0,96 in monomeric recessivity. In the gene ratio 1 : 9 : 9 : 1 the difference is

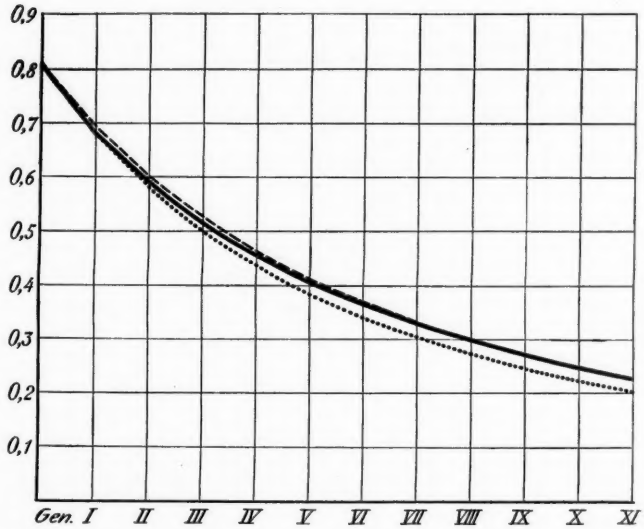

Fig. 42. Dimeric recessivity. Gene ratio 1 *A* : 9 *a* : 9 *B* : 1 *b*. Elimination of *aabb* (initial frequency 0,81 per cent). Signification of the curves, see Fig. 38.

quite unsignificant (less than 0,52 and 0,50 per cent, respectively), and in Gen. VII to Gen. X, where it attains its maximum, it has almost no practical importance (Gen. VIII: 0,30 and 0,274 per cent, respectively). In the last example the diminution is in Gen. IV 94,9 per cent of that in monomeric recessivity, and the difference is very slowly decreasing, in Gen. X 95,6 per cent. The gradually closer agreement with the effect obtained in monomeric recessivity is due to the prevailence of one of the two genes. The more frequent one of the genes is, *i. e.* the more conditions approach the case when it is present in all individuals, the greater is the role played by the other gene.

In this connection attention should be paid to the reduction of the frequency of the genes as illustrated in Figs. 41 and 43. The frequency of the rare gene *b* falls rather strongly and rapidly whereas that of the common gene *a* is but little affected, less in Fig. 43. The frequency of the genes, contrary to that of the character-carriers, is reduced less than in the theoretical case of mixing in each generation but the difference is not great; in Fig. 41 the mixing curve for *b* is drawn. — If selection stops, the frequency of *aabb* as in the preceding case slowly increases. The augmentation is, however, very slight, and is smaller the more the

Fig. 43. Dimeric recessivity. Gene ratio 1 *A* : 9 *a* : 9 *B* : 1 *b*. Frequency of the genes. Ordinate, frequency of *a* in per cent of *A*. Initial frequency of *b* augmented to the value of *a*.

one gene prevails. In the gene ratio 1 : 5 : 5 : 1 the increase after reaching of equilibrium is at most (in Gen. III) 7,8 per cent (from 1,02 to 1,10 per cent), in Gen. X 4 per cent (from 0,430 to 0,447 per cent). In the ratio 1 : 9 : 9 : 1 it is, in Gen. X, but 2,4 per cent (increase from 0,250 to 0,256 per cent).

DIMERIC RECESSIVITY-DOMINANCE.

The gene ratio 2 *A* : 1 *a* : 1 *B* : 2 *b* is of interest when compared with the first case of double recessivity (1 : 1 : 1 : 1), the initial frequency of the character-carriers *aaB*-, 6,17 per cent, being almost the same as in this case. Fig. 44 shows that the frequency is reduced more rapidly but that the difference is rather small and that the results on the whole coincide fairly well. The actual curve and the mixing curve converge from Gen. I, then (from Gen. VII) almost coincide (very slightly divergent). The actual curve and the curve for monomeric recessivity diverge at first (from Gen. I), then (Gen. V—VII) converge very slightly. Another example of the same type, the gene ratio 4 *A* : 1 *a* : 1 *B* : 4 *b* (initial frequency of *aaB*- 1,44 per cent) is illustrated in Fig. 46. The actual curve and the mixing curve meet later, after Gen. X. In these cases (and other of the same type) the actual effect in the first two

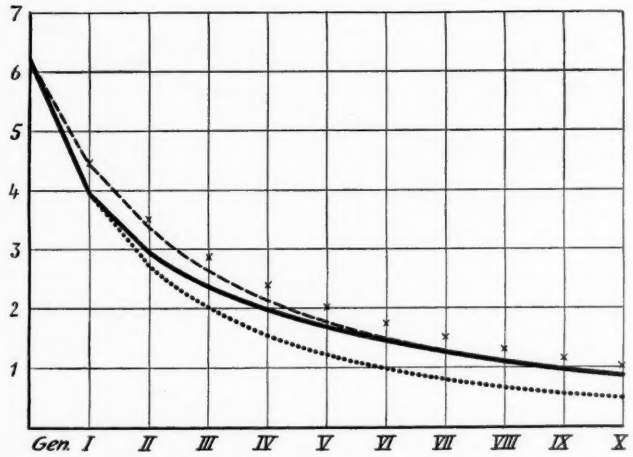

Fig. 44. Dimeric recessivity-dominance. Gene ratio 2 *A* : 1 *a* : 1 *B* : 2 *b*. Elimination of *aaB-* (initial frequency 6,17 per cent). Signification of the curves and crosses, see Fig. 38.

generations is the same or about the same as in monomeric recessivity or not much weaker (in Fig. 46, in Gen. I, a little stronger). The curves are then at first divergent, later (not in Fig. 46, begins after Gen. X) slightly convergent. From about Gen. IV the effect is markedly weaker than in monomeric recessivity. In Fig. 44 the reduced frequencies are in Gen. IV 90,5, in Gen. VII 91,1, in Gen. X 93,3 of those obtained in monomeric recessivity. In Fig. 46 the corresponding figures are 87,2,

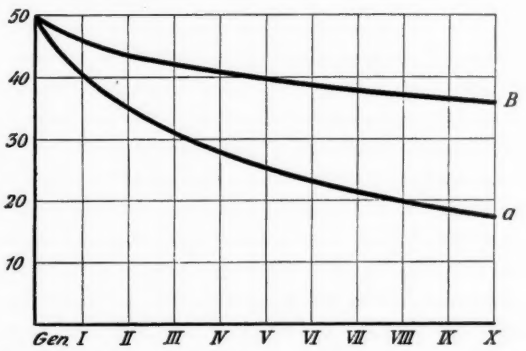

Fig. 45. Dimeric recessivity-dominance. Gene ratio 2 *A* : 1 *a* : 1 *B* : 2 *b*. Elimination of *aaB-*. Frequency of the genes; *a* in per cent of *A*, *B* in per cent of *b*.

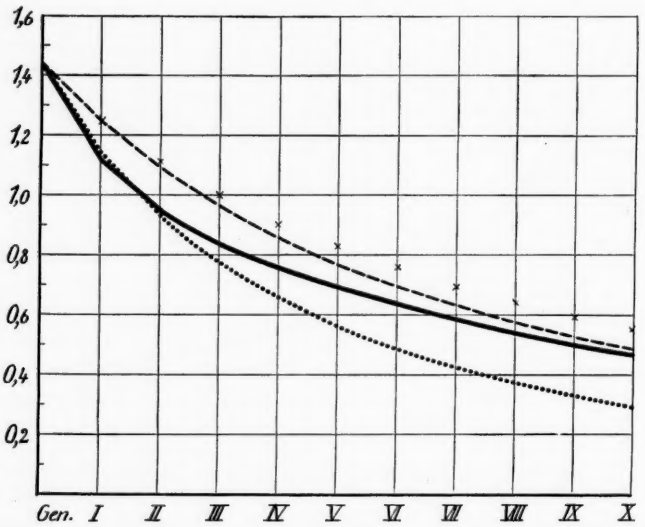

Fig. 46. Dimeric recessivity-dominance. Gene ratio 4 *A* : 1 *a* : 1 *B* : 4 *b*. Elimination of *aaB-* (initial frequency 1,44 per cent). Signification of the curves and crosses, see Fig. 38.

84,3, 84,9 per cent. The effect attained in the latter case in Gen. VI is attained only in Gen. IX to X.

The two genes in these cases have initially the same frequency. The reduction is, however, not the same by far but considerably stronger for the recessive gene (Figs. 45 and 47); the difference is somewhat decreased with decreased frequency of the genes. In monomerism the frequency of dominant genes is, as is well known, always much more rapidly reduced than that of recessive genes (in cases of complete dominance and total selection they are of course removed at once). In combined recessivity-dominance with equal frequency of the dominant and the recessive genes it is quite the opposite. This is due to the fact that in such dimerism the dominant genes occur to a larger extent in gene combinations where they are inactive and consequently not eliminated. In the simplest case, the gene ratio 1 *A* : 1 *a* : 1 *B* : 1 *b* (*aaB-* $^3/_{16} = 18{,}75$ per cent), in the initial generation $^3/_8$ *a* but only $^2/_8$ *B* (*i. e.* $^2/_3$ of the number of *a*) occur in genotypes where they manifest themselves. In the example 2 *A* : 1 *a* : 1 *B* : 2 *b* given above $^5/_{27}$ *a* but only $^3/_{27}$ *B* (*i. e.* $^3/_5$ of the number of *a*) manifest themselves and are eliminated.

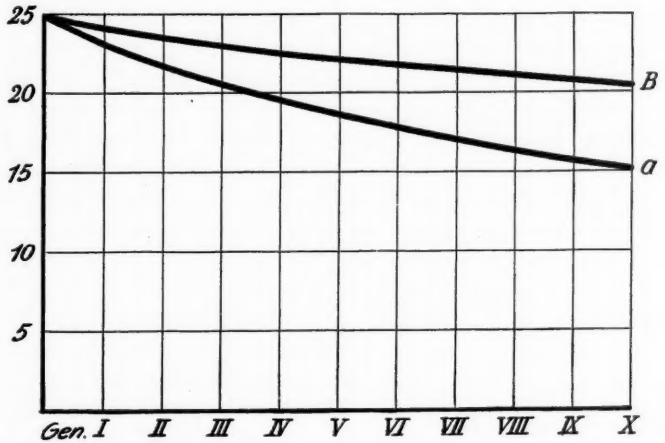

Fig. 47. Dimeric recessivity-dominance. Gene ratio 4 *A* : 1 *a* : 1 *B* : 4 *b*. Elimination of *aaB*-. Frequency of the genes, *a* in per cent of *A*, *B* in per cent of *b*.

As in cases of dimeric recessivity the reduction of the character-carriers is stronger than what is due to the reduced frequency of the genes. The position of the actual curve below the mixing curve in Figs. 44 and 46 lies up with this. The mixing curve for the frequency of the genes lies not above but below the actual curve but the difference is not great and the former curve is not drawn in Figs. 45 and 47. If elimination stops and equilibrium is slowly reached, the frequency of the character-carriers is increased and lies a little above that indicated in the mixing curve (the crosses in Figs. 44 and 46).

When the recessive gene is more or less common, the dominant gene more or less rare, the effect of selection, owing to the important role played by the dominant gene, is in several respects different. It is for the gene ratio 20 *A* : 20 *a* : 1 *B* : 39 *b* illustrated in Fig. 48. The actual effect is very strong and rapid. The actual curve and the mixing curve cross already in Gen. VI and are then distinctly divergent. As shown before (p. 230), the effect of the selection is from the beginning much stronger than in monomeric recessivity. In Gen. I it is more than 2 $^1/_2$ times (262 per cent) as strong; the difference then sinks and is in Gen. IV 141, in Gen. X 115 per cent of the effect in monomeric recessivity. The actual curve thus lies considerably below the curve for monomeric recessivity; the two curves will meet only in remote generations. If selection stops and equilibrium is slowly reached, the frequency of

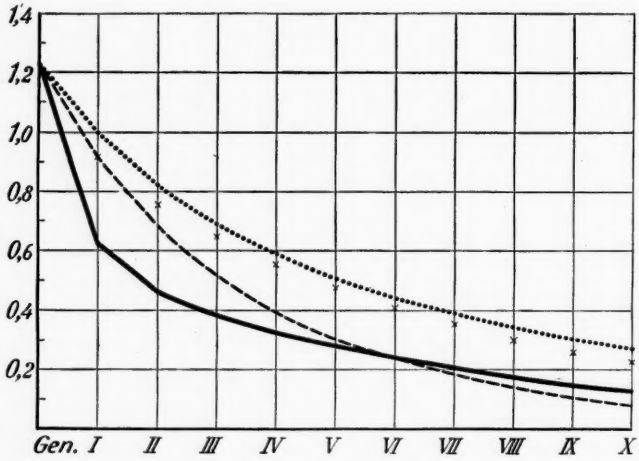

Fig. 48. Dimeric recessivity-dominance. Gene ratio 20 *A* : 20 *a* : 1 *B* : 39 *b*. Elimination of *aaB*- (initial frequency 1,234 per cent). Signification of the curves and crosses, see Fig. 38.

aaB-, as in the previous cases, is increased (the crosses in Fig. 48) but is still lower than in monomeric recessivity. The great size of the increase indicates that the effect of the elimination is much greater than what

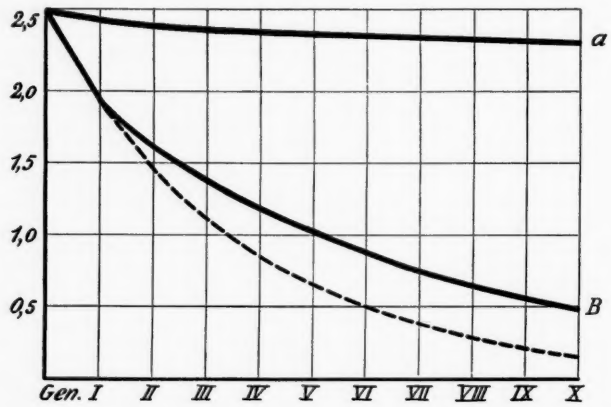

Fig. 49. Dimeric recessivity-dominance. Gene ratio 20 *A* : 20 *a* : 1 *B* : 39 *b*. Frequency of the genes. Ordinate, frequency of *B* in per cent of *b*. Initial frequency of *a* reduced to the value of *B* (2,56). Dashed line, mixing curve for *B*.

is due to the reduced frequency of the genes. This is seen also from the curves for the reduction of the frequency of the genes, Fig. 49: the imaginary mixing curve (drawn for *B* only) lies considerably lower than the actual curve. The diagram further shows that the dominant gene is strongly reduced, the recessive gene only very slightly, quite contrary to what occurs in cases such as those of Figs. 45 and 47. This is due, as is easily understood, to the preponderance of the dominant gene. There are, of course, a series of transitions between these two types of recessivity-dominance, for instance where the frequency of the dominant and of the recessive gene is about equally lowered.

If the recessive gene (*a*) is very common, *i. e.* much more common than the dominant allele (*A*), the dominant gene *B* still being rare as in the previous cases, the latter gene plays a still greater role and elimination has a very strong effect. The rarer the dominant and the more common the recessive gene, the more the relationships approach conditions where all individuals are *aa* (and all except the *B*-carriers *aabb*), *i. e.* monomeric dominance. In the gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b*, for instance, 81 per cent of the individuals are *aa*, 18 per cent *Aa*, and *a* is present in 90 per cent of the gametes; after elimination of the 15,39 per cent *aaB*- the frequency is in Gen. I 1,86 per cent, after reaching of equilibrium 3,45 per cent. In the gene ratio 1 *A* : 19 *a* : 1 *B* : 19 *b*, 90,25 per cent of the individuals are *aa*, 9,5 per cent are *Aa*, and 90 per cent of the gametes contain *a*; after elimination of the 8,80 per cent *aaB*- the frequency in Gen. I is only 0,49 per cent, in equilibrium (*cf.* Table 26) 0,95 per cent. In monomeric recessivity the reduced frequency in these two examples is 8,37 and 5,27 per cent, respectively. Curves for the reduction in these extreme cases approaching monomeric dominance would have no real interest.

There are, of course, extreme cases of an opposite kind, where the relation between the recessive and the dominant gene is reversed. They approach monomeric recessivity, the recessive gene being rare and the dominant gene frequent. In the gene ratio 8 *A* : 1 *a* : 8 *B* : 1 *b*, for instance, *B* is present in 98,8 per cent of the individuals and in 88,9 per cent of the gametes. Elimination causes almost exactly the same reduction of the frequency of the character-carriers as in monomeric recessivity: initial frequency of *aaB*- 1,22, Gen. I 0,99, Gen. II 0,82, Gen. III 0,69 per cent. Equilibrium causes practically no difference.

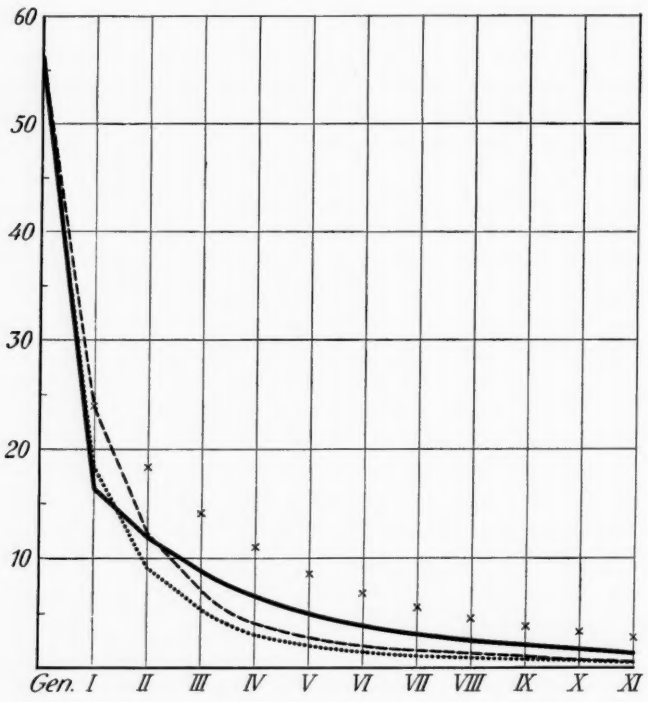

Fig. 50. Dimeric dominance. Gene ratio 1 *A* : 1 *a* : 1 *B* : 1 *b*. Elimination of *A-B-* (initial frequency 56,29 per cent). Signification of the curves and crosses, see Fig. 38.

DIMERIC DOMINANCE.

The simplest case 1 *A* : 1 *a* : 1 *B* : 1 *b* has no practical interest for man, the initial frequency of the character-carriers *A-B-* being very high, 56,25 per cent, but gives an instructive starting-point (Fig. 50). Elimination of *A-B-* is followed immediately by a mighty decrease of the character-carriers, to 16,3 per cent; the frequency then sinks at moderate speed. The mixing curve lies at first above the actual curve; the two curves then cross and diverge and after Gen. IV converge again. The curve for monomeric recessivity lies below the mixing curve, from Gen. IV very near it. The frequency of the genes is (after the first generation) less reduced as though mixing were presumed (Fig. 51). It follows, as in previous similar cases, that the frequency of the character-carriers rises, if elimination stops and equilibrium is slowly reached (the crosses in Fig. 50).

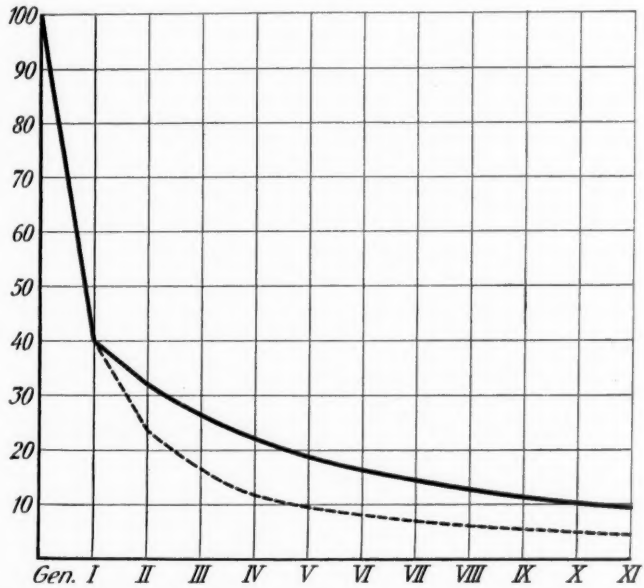

Fig. 51. Dimeric dominance. Gene ratio 1 *A* : 1 *a* : 1 *B* : 1 *b*. Elimination of *A-B-*. Frequency of the genes, *A* in per cent of *a* (and *B* in per cent of *b*). Dashed line, mixing curve.

If the frequency of *A* and *B* is lower, the effect is principally equal but the point where the curves cross is postponed. In the gene ratio 1 *A* : 2 *a* : 1 *B* : 2 *b*, initial frequency 30,9 per cent, the difference from Fig. 50 is slight but the three curves lie very near each other (Fig. 52). In the gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b*, initial frequency 3,6 per cent, the actual curve and the mixing curve meet only in Gen. VIII (Fig. 53). The latter curve and the curve for monomeric recessivity are nearly coincident. Though the frequency of the character-carriers in several generations is considerably more decreased than if mixing of the genes is supposed, the frequency of the genes is less reduced than in the latter case (Fig. 54). If elimination stops and equilibrium is at last reached, the frequency of *A-B-* is rather much increased (to 2,25 per cent in Gen. II). In the ratio 1 *A* : 19 *a* : 1 *B* : 19 *b*, initial frequency 0,95 per cent, the curves cross after Gen. X (Fig. 55). The mixing curve and the curve for monomeric recessivity are so nearly coincident that the latter could hardly be drawn in the diagram (distance about 0,005 per cent). The curves

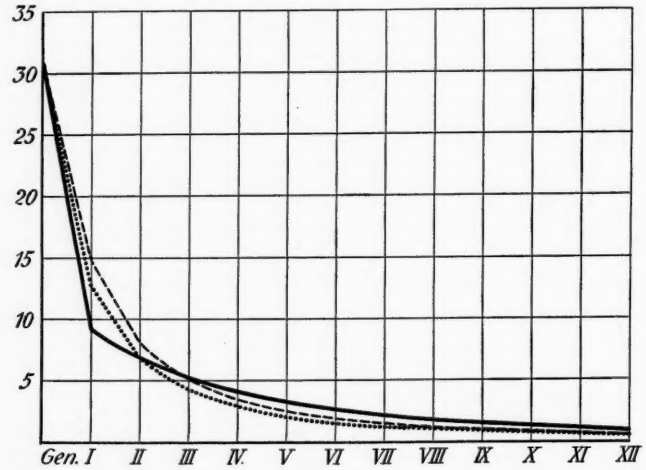

Fig. 52. Dimeric dominance. Gene ratio 1 *A* : 2 *a* : 1 *B* : 2 *b*. Elimination of *A-B-* (initial frequency 30,9 per cent). Signification of the curves, see Fig. 38.

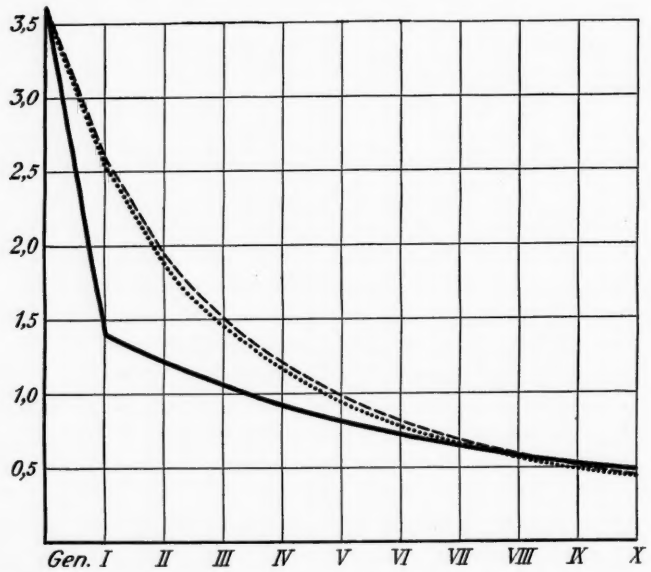

Fig. 53. Dimeric dominance. Gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b*. Elimination of *A-B-* (initial frequency 3,61 per cent). Signification of the curves, see Fig. 38.

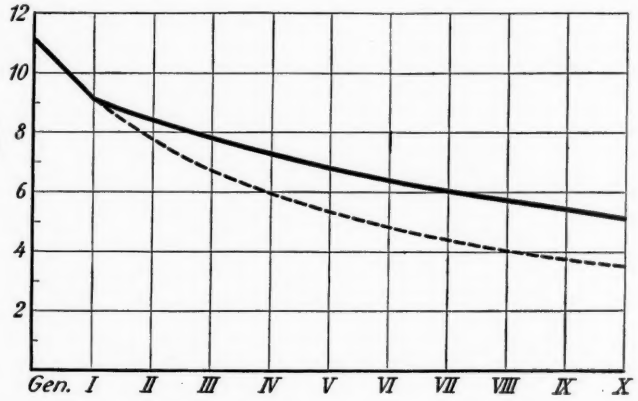

Fig. 54. Dimeric dominance. Gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b*. Elimination of *A-B-*. Frequency of the genes, *A* in per cent of *a* (and *B* in per cent of *b*). Dashed line, mixing curve.

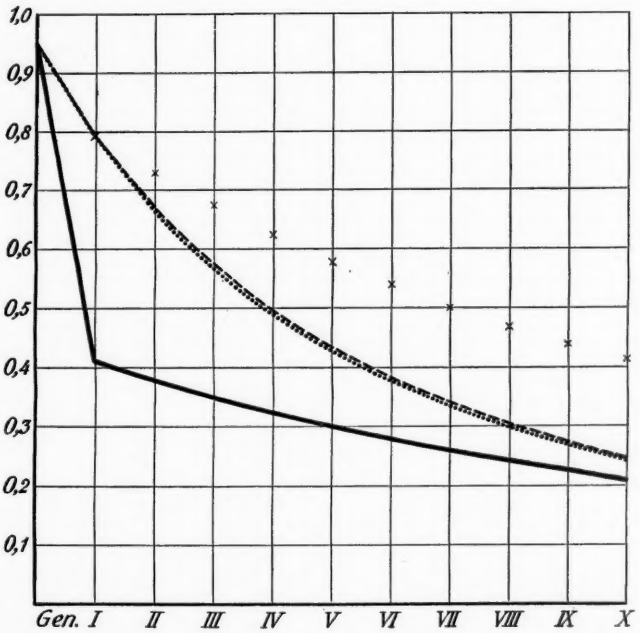

Fig. 55. Dimeric dominance. Gene ratio 1 *A* : 19 *a* : 1 *B* : 19 *b*. Elimination of *A-B-* (initial frequency 0,950 per cent). Signification of the curves and crosses, see Fig. 38.

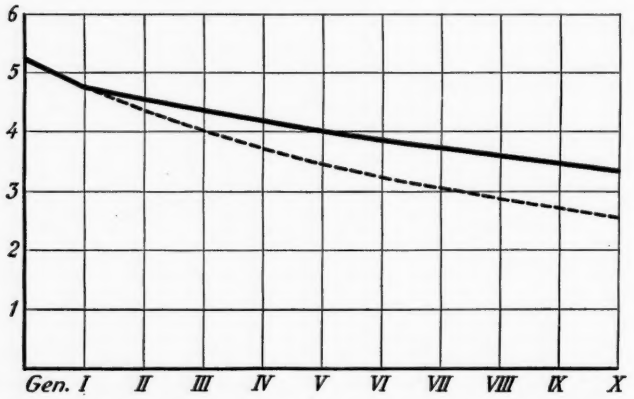

Fig. 56. Dimeric dominance. Gene ratio 1 *A* : 19 *a* : 1 *B* : 19 *b*. Elimination of *A-B-*. Frequency of the genes, *A* in per cent of *a* (and *B* in per cent of *b*). Dashed line, mixing curve.

for the genes are given in Fig. 56. If elimination stops and equilibrium is reached, the frequency of the character-carriers is considerably increased (the crosses in Fig. 55).

TRIMERISM.

The variations are, as was remarked before (p. 233), very strong. I give only two extreme examples. In trimeric recessivity with equal frequency of the genes the initial frequency of the character-carriers (1,56 per cent) after the first generation is considerably less reduced than in monomeric recessivity and the difference increases gradually; the reduction is in Gen. II 83,9, in Gen. III 76,3, in Gen. IV 70,8 per cent of the diminution in monomeric recessivity (Fig. 57). The reduction of the character-carriers, though rather limited after the first generation, is yet greater than what is due to the frequency of the genes. If elimination stops and equilibrium is established, the frequency of *aabbcc* is considerably increased (the crosses in Fig. 57).

In trimeric dominance with the gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b* : 1 *C* : 9 *c* the frequency of the character-carriers is at first strongly reduced, much more than in monomeric recessivity (Fig. 58). The difference is then diminished; the curves meet in Gen. V and then diverge slightly. As in similar cases of dimerism the strong reduction in the first generations concerns the individuals, not the genes. If elimination stops, the

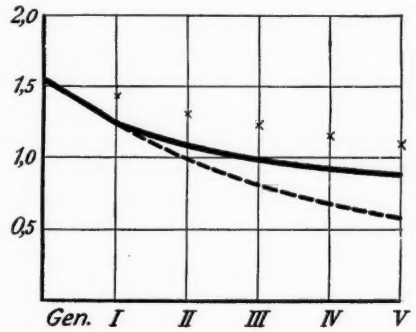

Fig. 57. Trimeric recessivity. Gene ratio 1 *A* : 1 *a* : 1 *B* : 1 *b* : 1 *C* : 1 *c*. Elimination of *aabbcc* (initial frequency 1,56 per cent). Dashed line, monomeric recessivity (mixing curve not drawn), crosses, see Fig. 38.

frequency of the character-carriers increases and is in equilibrium higher than the corresponding frequencies in monomeric recessivity (the crosses in Fig. 58).

CONCLUDING REMARKS.

The preceding account shows that in cases of di- and trimerism elimination of the character-carriers causes a reduction of their frequency which in the first generation is either the same as in monomeric recessivity (in cases of double and triple recessivity and certain cases of mixed recessivity-dominance) or somewhat stronger (certain cases of recessivity-dominance and of double dominance) or much stronger

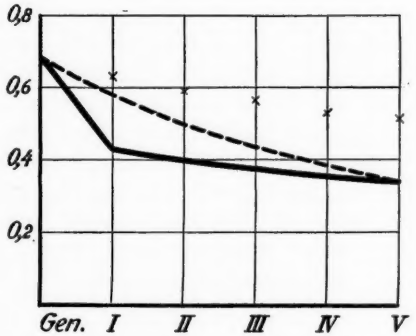

Fig. 58. Trimeric dominance. Gene ratio 1 *A* : 9 *a* : 1 *B* : 9 *b* : 1 *C* : 9 *c*. Elimination of *A-B-C-* (initial frequency 0,686 per cent). Dashed line, monomeric recessivity (mixing curve not drawn), crosses, see Fig. 38.

(most cases of double and triple dominance, extreme cases of recessivity-dominance). In subsequent generations the effect is often weaker than in monomeric recessivity but the difference is in low frequencies mostly more or less insignificant (Figs. 40 and 42). In cases of double and triple dominance where the effect is primarily very strong it remains in several generations stronger than in monomeric recessivity and is later about the same (Figs. 53 and 58). In triple recessivity the effect after the first generation is considerably weaker than in monomeric recessivity and the difference increases throughout many generations (Fig. 57).

The actual effect of elimination is in the first generations, usually in a relatively great number of generations, stronger than what is due to the reduced frequency of the genes. The elimination brings on a state where equilibrium between the genes is disturbed; a more or less great part of the genes which, when united, produce the character are to a greater extent than before the elimination separated and inactive, there is in other words a deficit of the character-carriers (for further particulars, see dimeric recessivity, p. 238). This fact has two consequences. If a complete mixing of the genes could occur in each generation, the curve for the reduction of the frequency of the character-carriers would lie above the actual curve (Figs. 38, 40, 44, 45, *etc.*), whereas the relation of the corresponding curves for the genes is reversed (Figs. 39, 41). Further, when elimination ceases, the frequency of the character-carriers slowly increases and lies, when equilibrium is at last reached, markedly above the frequency resulting from the elimination. The difference is in some cases rather great in the first generations, in most cases more or less moderate; in later generations it is usually moderate.

From a practical point of view, in cases where the effect is not constantly about the same or only a little weaker than in monomeric recessivity (Figs. 40, 42, 44, 50, 52) or in many generations even stronger (Figs. 48, 53, 55), stress must be laid upon the relatively strong effect in the very first generations or at least in the first generation. In the case illustrated in Fig. 46, for instance, the effect after a few generations is considerably weaker than in monomeric recessivity but in the two first generations about the same (in the first one even a little stronger). The possibilities for a reduction of the frequency of hereditary defects by means of elimination of the character-carriers from reproduction — in most cases very small or none — will not be discussed in this paper. It may be remarked, however, that if a reduction is possible, the results obtainable in the first generations have from a

practical point of view the greatest importance. A reduction in the first or the first two or three generations would in any case, if maintained, be a valuable result. The importance of the very first generations is not inconsistent with the fact that in di- and polymerism the reduction of the character-carriers is not matched by an equally strong reduction of the genes and thus to a certain degree is phenotypical. If a certain percentage of a gene which in a special gene-combination is responsible for a defect is continually inactive, this percentage of the gene is harmless. (An important reservation must be made: the gene itself may cause a minor defect.)

If selection stops, the balance between genes and genotypes is gradually restored and the number of the character-carriers consequently increased. This rise is, however, very slow and, in frequencies being of practical interest in man, rather slight.

If in di- or polymerism one of the genes is recessive and sex-linked, the effect of selection is increased. The conditions are fairly complicated and calculations laborious. If the sex-linked gene plays a main role, the effect will be strong, in other cases it is but moderately increased. I shall possibly have reason to touch upon this subject in a future paper dealing with some cases of a possible selectional effect.

MUTATION AND SELECTION.

In the preceding sections the theoretical effect of selection by means of elimination of the character-carriers is discussed under the presumption that no increase of the genes occurs due to mutation. If new genes arise at the same time as the frequency is decreased by selection, the latter process has of course a weaker effect than if we have merely to consider the genes existing when the elimination sets in. To estimate the influence of mutation on the effect of selection a basis exists which in principle is perfectly reliable. As HALDANE (1927, 1936, 1940, 1941) has shown, mutation and selection, if their rates and other factors are unaltered, will come into equilibrium; sooner or later, which in most cases is very late, a state is attained in which just as many genes are eliminated as arise by mutation. The establishment of this fact, simple as it may seem, is, according to my opinion, one of the most important contributions to modern population genetics.

I should like to intercalate a few remarks which, though really self-evident, may be of some use in understanding the following.

If equilibrium exists, of course only a change in the intensity of

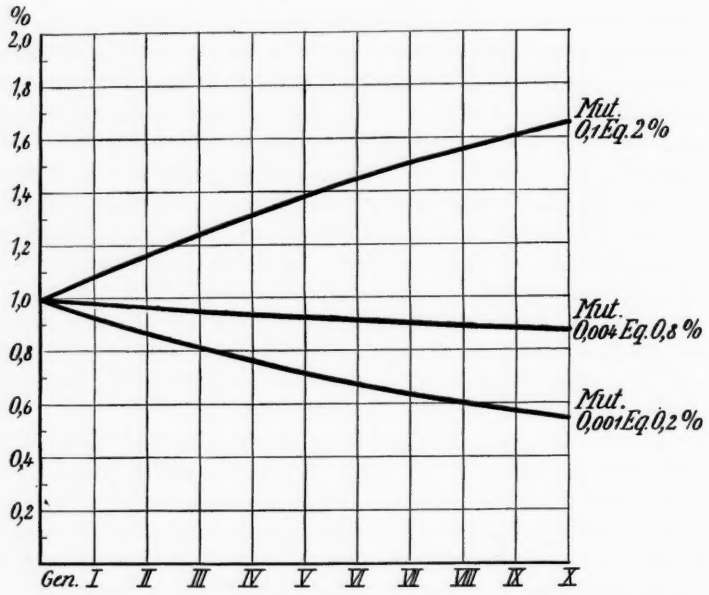

Fig. 59. Monomeric autosomal recessivity. Initial frequency of the recessive homozygotes (panmixia) 1 per cent. Negative selection 0,5. Mutation rates (theoretical values, partly preposterously high; *cf.* the text) 0,1, 0,004 and 0,001.

selection or mutation, or of both processes, or of the breeding system, can cause a change in the relative frequency of genes and character-carriers (apart from changes by chance in small populations). What is of interest in this connection is a state in which equilibrium is not established; it may have been disturbed or never have existed. Let the point of starting be a population in which, as far as a certain gene is concerned, neither mutation nor selection occurs — of course a purely theoretical assumption. If mutation only sets in, the mutant gene will in the long run completely displace its allele. On the other hand, if only selection disfavouring the same gene works, the gene will at last be totally (or in recessivity almost totally) removed from the population. Theoretically, there is a series of continuous transitions between these two extremes. It begins with an equilibrium immediately below a frequency of the gene (or character-carriers, if their frequency be regarded) of 100 per cent; this state is established when the mutation rate is more or less high, the selection insignificant. The series ends where the fre-

quency is approaching zero; this state is established when the mutation rate is very low, the selection vehement. What has real interest is, of course, not such extreme theoretical cases but cases where the differences are more moderate.

The equilibrium frequency is independent of the initial frequency. This fact may in spite of its obviousness be exemplified by a diagram (Fig. 59); I give it somewhat hesitatingly but it may be of some use in clarifying the whole matter. The mutation rates assumed are unnaturally high, in the case of the uppermost curve preposterously high, but the diagram has the sole purpose of illustrating the principles, which are alike in lower frequencies and lower mutation rates. It holds good for monomeric recessivity but applies in principle to all types of inheritance. The initial frequency of recessive homozygotes in the diagram is high, 1 per cent, and the selection in the three cases the same, 0,5. The curves illustrate the result in the first ten generations for three different rates of mutation, 0,1, 0,004 and 0,001. The new equilibrium frequency lies in the first example, in which the frequency increases, at 2 per cent and will be attained comparatively quickly; in the second case the equilibrium, 0,8 per cent, is approached very slowly, and in the third case equilibrium, 0,2 per cent, will be reached very late.

MONOMERIC AUTOSOMAL RECESSIVITY.

In Figs. 60—62 three examples are given in which, for three different initial frequencies of recessive homozygotes, 2, 1 and 0,01 per cent, equilibrium between a negative selection of 0,5 and mutation is presumed. In the two first examples this equilibrium would require a quite impossibly high mutation rate, 0,01 and 0,005, respectively. The examples are, in other words, unnatural; in such high frequencies a strong selection can never be balanced by mutation. The examples illustrate fairly well, however, the principles and the differences between different frequencies; the calculations are easier to make than in very low frequencies.

It is assumed that a total elimination of the character-carriers sets in. A glance at the diagrams shows that the reduction of their frequency is about half of what it would have been without mutation (the dashed curves). The frequency will continue to sink after the tenth generation until a new equilibrium corresponding to total selection ($r^2 =$ mutation rate) is reached.

In the high frequency of 2 per cent r^2 (Fig. 60) the reduction is in the first generation 51,61 of the reduction without mutation. This value

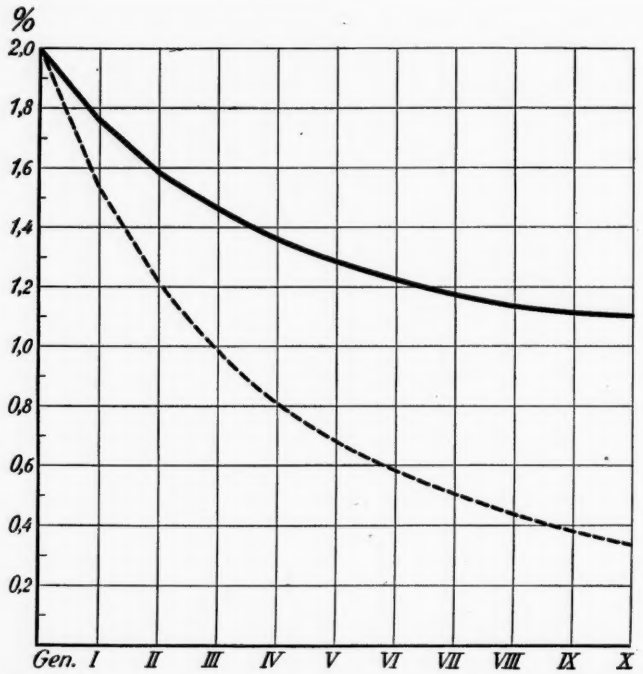

Fig. 60. Monomeric recessivity. Initial frequency 2 per cent; equilibrium between negative selection of 0,5 and »mutation» = »mutation» 0,01. Effect of total elimination of the recessive homozygotes. Dashed line, decrease without mutation.

rises to about 55,2 per cent in Gen. VIII. The frequency is in Gen. X 1,09 per cent; equilibrium, which lies at 1 per cent, is thus almost attained. The non-mutation curve will of course continue to sink to (practically) zero. The figure 55,2 will, in other words, gradually sink to 50.

In 1 per cent frequency (Fig. 61) the reduction is a little less strong in comparison to what occurs without mutation but the difference is small: 51,18 per cent in Gen. I rising to 54,6 per cent in Gen. X. The frequency is in this generation reduced to 0,59 per cent. Equilibrium, 0,5 per cent, is strongly approached but will be reached much later than in the former case.

The last example, initial frequency 0,01 per cent (Fig. 62), is a little more natural, though the mutation rate (1 : 20.000) is higher than what is likely to occur in man. The reduction is in Gen. I 50,14 per cent of the

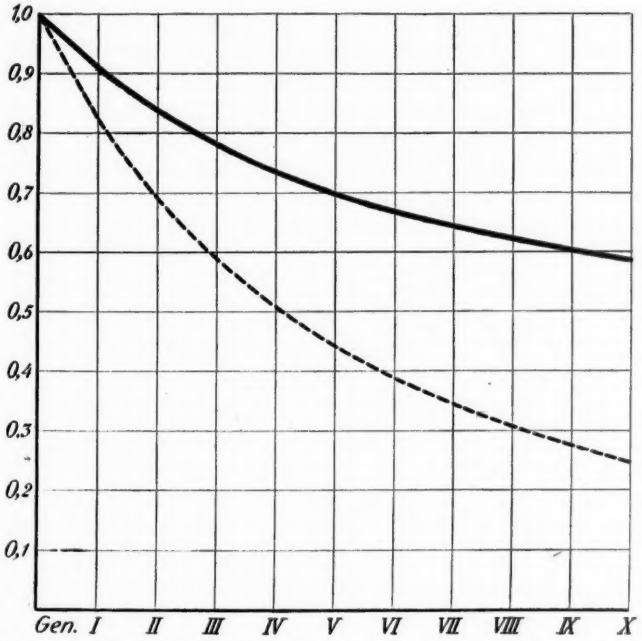

Fig. 61. As Fig. 60 but initial frequency 1 per cent, »mutation» 0,005.

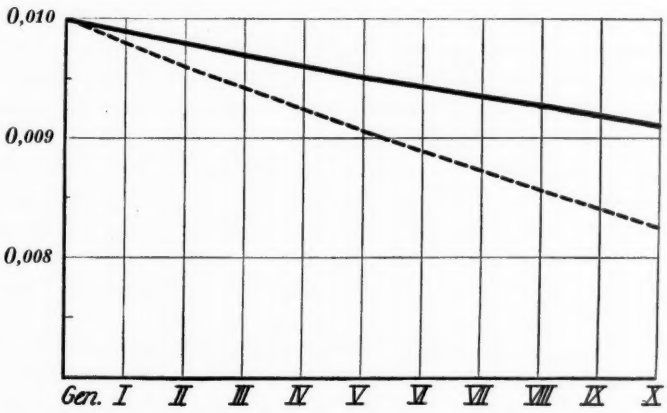

Fig. 62. As Fig. 60 but initial frequency 0,01 per cent, mutation 0,00005.

TABLE 30. *Monomeric autosomal recessivity. Initial equilibrium between selection and mutation. Effect in Gen. I of total elimination of the recessive homozygotes as compared with the effect in panmixia without mutation and previous equilibrium. Frequencies in per cent. Figures in italics: reduction in per cent of the reduction obtained in cases without mutation (for the calculations more decimal places were taken than included in the table).*

Initial frequency		2		1		0,1		0,01	
No mutation		1,535	—	0,83	—	0,094	—	0,0098	—
Initial equilibrium	Selection $^1/_{10}$	1,58	*90,59*	0,84	*90,38*	0,0946	*90,13*	0,00982	*90,05*
	Selection $^1/_4$	1,645	*76,24*	0,87	*75,89*	0,0955	*75,28*	0,00985	*75,05*
	Selection $^1/_2$	1,76	*51,61*	0,91	*51,18*	0,0970	*50,38*	0,00990	*50,14*
	Selection $^3/_4$	1,88	*26,27*	0,955	*25,81*	0,0985	*25,42*	0,00995	*25,10*

reduction in a case without mutation and the figure rises slowly, to about 50,6 in Gen. V. The frequency is in Gen. I reduced from 10 to 9,9 per 100.000, in Gen. V to 9,5 and in Gen. X presumably (no exact calculation is made) to a little more than 9,1. Equilibrium, 5 per 100.000, will not be reached until after many hundreds of generations, when in a case with no mutation the recessive homozygotes are practically removed. After 100 generations the frequency, about 6,25 per 100.000, still lies a fair way above the equilibrium value.

In other rates of mutation balanced by selection corresponding results are obtained. As is easily understood, the effect of total elimination decreases gradually with increasing values for the mutation—selection. When mutation (and previously balanced selection) is insignificant, the effect of total selection is almost the same as in cases without mutation; when mutation is very strong, the reduction is strongly diminished. The regular drop in the effect is illustrated in Table 30 for four frequencies and four equilibrium values. The table gives the result in the first generation (about the subsequent generations, *cf.* above; calculations in the other cases would be of little interest). (The high frequencies with unnatural mutation rates come under the same postulation as before.)

For the four equilibrium values given in the tables (selection $^1/_{10}$, $^1/_4$, $^1/_2$ and $^3/_4$) the reduction is reduced to about 90, 75, 50 and 25 per cent, respectively. The connections are very simple: if in equilibrium the balanced selection were k (or $1 - f$), the reduction is in low frequencies

$1 - k$ (or f) of the reduction without mutation. In higher frequencies (where the mutation rate would be impossibly high) the values rise but slightly.

SEX-LINKED RECESSIVITY.

In autosomal recessivity HALDANE's formula for equilibrium between selection and mutation, as well as other corresponding formulae, implies that if a population, mating at random, be in equilibrium and selection and mutation according to the formula set in, the composition of the population will remain unaltered. The formula given by HALDANE for sex-linked recessivity, $\mu = {}^1/_3\ (1 - f)\ x$, has not this significance. If a population is in genetical equilibrium, generally as well as between the sexes, it never remains unaltered, if both selection and mutation set in; if HALDANE's formula be employed, it appears as if the gene frequency is sinking. The cause is that selection affects mainly or in low frequencies nearly exclusively the males, whereas mutation affects both sexes. The relation between the genes of the males and of the females, initially $1\ ♂\ r : 2\ ♀\ r$, is from the first generation — if the unnatural supposition of sharply limited generations be made — altered to a surplus of males and a deficit of females. After a few generations an equilibrium is reached. HALDANE's formula is valid when this equilibrium is taken as starting point. This equilibrium is dependent on the intensity of the selection but independent of the frequency of the genes. If the frequency of the gene (and character) in the males be 1, the female gene frequency is in equilibrium $2 - 2/3\ (1 - f)$ or, if $1 - f$ is substituted by a letter for the rate of selection, for instance k, $2 - 2/3\ k$. Therefore, if the selection rate is ${}^3/_4$, the distribution of the genes in equilibrium is given by $1\ ♂\ r : 1{,}5\ ♀\ r$, if selection is ${}^1/_2$, the distribution is $1\ ♂\ r : 5/3\ ♀\ r$, *etc.* If we begin, not from this state but from a population in panmixia equilibrium, $1\ ♂\ r : 2\ ♀\ r$, the equilibrium attained can be calculated according to the following formula, in which μ is the mutation rate:

$$\mu = \frac{1}{3 - 2/3\ k} \cdot kr^2.$$

I owe this formula to Professor I. SPERBER, with whom I have discussed this problem. It is not valid for high frequencies.

What should now be considered is the effect of total elimination of male character-carriers in cases of previous equilibrium between partial selection and mutation, as compared with the effect obtained when the starting point is represented by a population in which neither selection

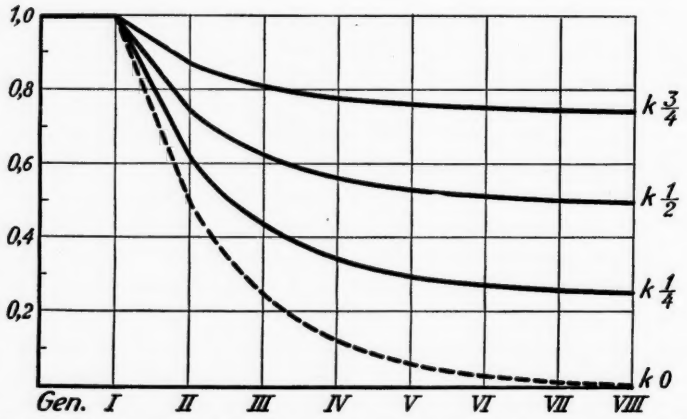

Fig. 63. Sex-linked recessivity. Initial equilibrium between selection and mutation. Total elimination of the male character-carriers sets in. Effect in 8 generations as compared with the effect in cases without previous equilibrium between partial selection and mutation (dashed line). *k*, selection in the initial equilibrium.

nor mutation occurs. A diagram is given in Fig. 63. The figures of the ordinate may signify frequencies in any arbitrary number, not too great; the initial frequency 1 may thus signify 1 per cent, 1 per 1000, 1 per 10000, *etc.* Three different rates of initial selection balanced by mutation are assumed: selection $^3/_4$, $^1/_2$, $^1/_4$. If the initial frequency is 1 per cent, the balancing mutation rates would be impossibly high, 1/400, 1/600 and 1/1200, respectively. If an initial frequency of 1 per 100.000 be assumed, the mutation rates giving equilibrium are 1/400.000, 1/600.000 and 1/1.200.000, respectively. The calculations are made on the basis of an initial frequency of 1 per cent. They are not absolutely exact, owing to the relatively high frequency of female character-carriers (0,0069 per cent in $^3/_4$ selection) which are not supposed to be subject to selection, but the deviations are insignificant and would not be visible in the diagram (the initial frequencies 1 and 0,01 per cent are, for instance, in $^3/_4$ selection reduced in Gen. III to 0,810 and 0,00812 per cent, respectively).

As in cases without previous selection and mutation, the frequency remains unaltered in the first generation; only an almost imperceptible reduction occurs (in $^3/_4$ selection to 0,9981, in $^1/_2$ selection to 0,9986, in $^1/_4$ selection to 0,9992 per cent). The reduction beginning in the next generation is, of course, dependent on the rate of mutation and previous selection: the feebler the mutation (and previous selection), the stronger is the reduction obtained through total elimination of the character-

carriers. The reduction continues only until a new equilibrium corresponding to total elimination is reached. This happens relatively quickly, in Gen. VII or VIII or a little later. There is some difference between different mutation rates but it is very slight. In $^3/_4$ previous selection the new equilibrium, at 0,75 per cent, is attained in Gen. VII; in $^1/_2$ selection equilibrium, at 0,5 per cent, is reached in Gen. VIII. In $^1/_4$ selection, where equilibrium lies at 0,25 per cent, the frequency in Gen. VIII is still a little higher, 0,255 per cent.

All this, by closer consideration, is rather self-evident. The most important conclusion is that if in a population in panmixia, total negative selection of the character-carriers sets in, the effect is very much dependent on the nature of the initial frequency; if this frequency has resulted from equilibrium between strong selection and mutation, the effect will be much feebler than what would be expected from a calculation on the initial frequency. Without mutation a frequency of 1 per 100.000 would, for instance, be reduced in 8 generations to practically zero (less than 1 in 10 millions); supposing previous balance between $^3/_4$ selection and mutation the frequency will only be reduced to 75 per cent of the initial frequency and not sink further.

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INCONSTANCY OF THE HUMAN SOMATIC CHROMOSOME COMPLEMENT

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I. INTRODUCTION.

IT is remarkable how little attention has been paid to animal chromosomes and nuclei outside the germ line. This neglect of the study of the somatic chromosomes seems in the first place to depend on the unshakable belief in the doctrine expressed in all text-books of cytology that all cells of a given organism contain the same somatic number of chromosomes. It is understandable that the necessity to stress the importance and continuity of the chromosomes induced O. HERTWIG in 1918 to express this idea in his »law of the constancy of chromosome number». It is less easy to understand, however, that so few attempts to check the validity of this law have been made even later, though a great number of findings are in opposition to it. We need only recall the polyploid tissues and cells found in animals (*cf.* GEITLER, 1938; OKSALA, 1939) as well as in plants (HUSKINS, 1949) to become aware of this fact.

In the present study we have examined somatic mitosis in man both in embryonic and adult tissues and found that the chromosome numbers vary from quite low hypoploids to polyploids, various aneuploid numbers being the most common. In the present paper only the outlines of this phenomenon are given; the studies are being continued in detail on various tissues. On the present evidence alone, however, it seems obvious that the prediction of OKSALA (1939, p. 143) »*. . . dass die somatische Polyploidie in gewisser Beziehung zum Differenzierungsprozess der Zellgewebe steht*» is valid in regard to certain tissues as such and may be extended in regard to others, in that various deviations of the normal chromosome complement and the normal course of mitosis certainly play a rôle in differentiation.

II. MATERIAL AND METHODS.

The chromosomes in adult man were studied in the proliferative stage of the uterine epithelium. Freshly excised biopsy specimens were

fixed in acetic alcohol (1: 3) for 1—2 hrs., stained in bulk in Feulgen and thereafter made into squash preparations (DARLINGTON and LA COUR, 1947). This method gave excellent results with adult chromosomes.

The embryonic chromosomes present much greater technical difficulties. This depends in part on the fact that the water content of embryonic tissues is very high, which seems to present an obstacle to satisfactory fixation. In part the chromosomes are actually sticky, which is proved by the occurrence of chromatid bridges in anaphases. The smaller the embryo, the more difficult its tissues are to fix.

The material consisted of tissues of 14 human embryos of both sexes aged from 6 to 24 weeks, which were fixed immediately after operation. To overcome the difficulties in fixation a great number of fixing and staining methods were tried. In the following table the various fixing and staining combinations are given together with the abbreviations used (for the formulas, see DARLINGTON and LA COUR, 1947).

Fixatives.

Stains	Lewitsky	Susa	2BD	Champy-Koller (K)	Ac. Alcohol (AA)
Feulgen (F)	+	+		+	+
Crystal violet (C) ..			+	+	
Haematoxylin				+	

The Feulgen-squash method did not give good results with the embryos. Fixation in Champy-Koller (2 hrs.) with a subsequent staining in crystal violet gave the best preparations. The colour was intensified by an after-treatment in 1 % chromic acid. The paraffin sections were 15 μ thick. Magnification of the camera lucida drawings: $\times$ 3000, that of the photomicrographs: $\times$ 1600.

III. OBSERVATIONS.

Our studies on the cytology of human cancer tissues (TIMONEN and THERMAN, 1950; THERMAN and TIMONEN, 1950) induced us to examine the chromosomes in the normal tissues of man. To obtain material of normal human mitosis for comparison we studied the uterine epithelium in its proliferative stage (TIMONEN and THERMAN, 1950). It was established that the cells displayed a great range of variation in chromosome number. This phenomenon has been investigated in

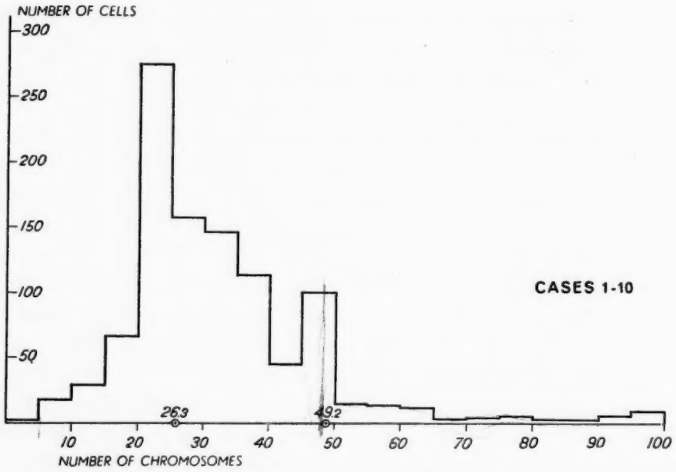

Fig. 1. Chromosome count made from one thousand cells of the human uterine epithelium in its proliferative stage (from TIMONEN, 1950).

further detail by TIMONEN (1950). He counted the chromosome number in one thousand cells of the uterine epithelium, and the result is shown in the adjoining diagram (Fig. 1). It is seen that the chromosome numbers vary from four to about one hundred, the highest peak lying at 20—25 chromosomes and a much lower one at 45—50 chromosomes.

This result was verified by measurements of the volumes of corresponding resting nuclei. The volumes obtained show a remarkable agreement with the chromosome numbers. The frequency peaks correspond with those shown by the chromosome numbers, and the nuclear volumes show the same range of variation.

To find out whether these observations had a more general applicability, we examined cytologically various tissues of human embryos. It could in fact be established that the same variation of the somatic chromosome number was characteristic of all the tissues studied, *viz.* skin, connective tissue, brain, liver, intestine, cartilage, and bone marrow.

As described in the previous chapter, the fixability of the embryonic chromosomes was not so good as that of the adult chromosomes. Chromosome counts could therefore not be performed to the same extent as in the uterine epithelium. Nor were the counts as exact. In those metaphase plates in which stickiness of the chromo-

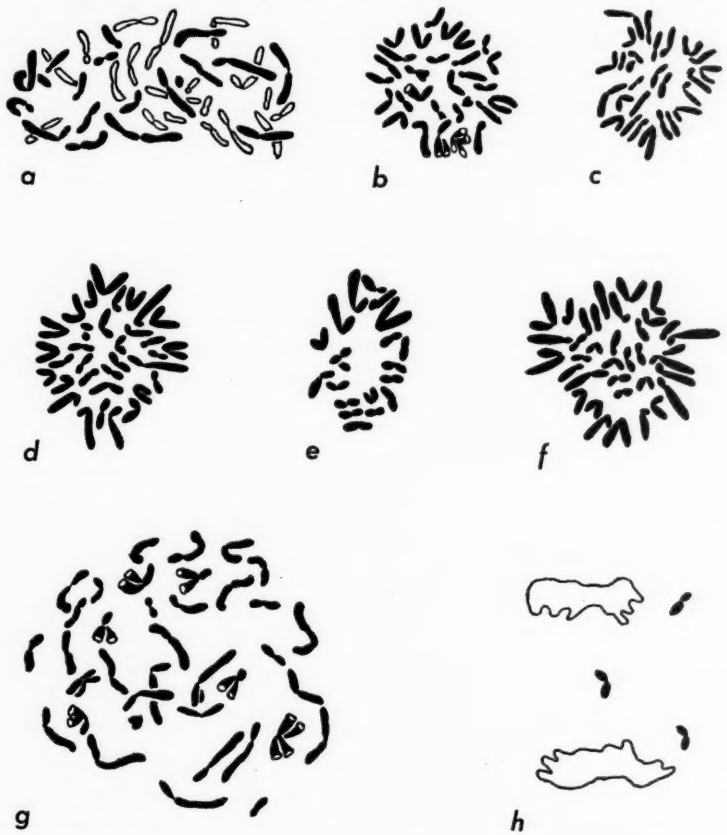

Fig. 2. Mitotic stages in human embryonic cells. — *a*: prophase with 37 chromosomes from connective tissue (8 weeks' embryo); *b*: metaphase with 29 chromosomes from connective tissue (8 weeks' embryo); *c—f*: metaphase stages from brain cells (8 weeks' embryo); *c*: 27 chromosomes; *d*: 36 chromosomes; *e*: 20 chromosomes; *f*: 35 chromosomes: *g*: prophase with 38 chromosomes from a liver cell (24 weeks' embryo); *h*: anaphase with laggards from connective tissue (8 weeks' embryo). (*g*: AA, F, the others K, C.)

somes rendered an exact count impossible the number could, however, in many cases be estimated, the sizes of the metaphase plates alone showing that most of the cells were hypoploid.

In addition to the uterine epithelium the brain cells of embryos gave the best preparations, and these cells were therefore studied more

extensively than other tissues. Figs. 2 *c*, *d*, *e* and *f* illustrate embryonic brain cells containing 27, 36, 20 och 35 chromosomes, respectively. In Fig. 3 *e*, again, we see a photomicrograph of the plate drawn in Fig. 2 *c*. It may be mentioned that not a single brain cell with exactly 48 chromosomes was encountered. Fig. 2 *a* represents a prophase in an embryonic cell from connective tissue with 37 chromosomes, while Fig. 2 *b* illustrates a metaphase plate with 29 chromosomes from the same kind of tissue. In Fig. 2 *g* an example of an embryonic liver cell with 38 chromosomes is given.

Owing to their better fixation photomicrographs could better be taken of the adult chromosomes. In Fig. 3 *a* a prophase with 48 chromosomes from the uterine epithelium is seen. Fig. 3 *b*, again, represents a prophase from the same tissue with 16 chromosomes only. In Fig. 3 *c* a metaphase stage with 40 chromosomes, also from uterine epithelium, is depicted. The photograph in Fig. 3 *d* has also been taken from a squash preparation of the uterine epithelium. It shows an epithelial metaphase with 44 chromosomes together with two anaphase groups with *ca.* 20 chromosomes each. This anaphase belongs to a lymphocyte cell, which seems to linger in a permanent anaphase. This phenomenon will be considered in detail elsewhere. In this connection only the low chromosome number characteristic of these lymphocytes is of interest.

The youngest embryos examined by us were 6 weeks old. Though a number of irregularities were observed in the mitoses it is not clear whether these, in themselves, are able to bring about the variation in the chromosome number prevailing in human tissues. To throw light on this point an investigation on rat embryos is planned, as in these animals the age of the embryos can be exactly controlled. This project is based on the assumption that variation in the somatic chromosome numbers will be found in all mammals at least.

It is known that normal mitosis consists of a number of fairly independent processes, *viz.* intrachromosomal changes and extrachromosomal changes, which act strictly in step. Timing differences between these bring about various modifications of mitosis, the most important of which is meiosis (DARLINGTON, 1937). In meiosis the extrachromosomal mechanism is precocious as compared with the intrachromosomal changes (DARLINGTON, 1937; OKSALA, 1944). As pointed out by OKSALA (1944), a great number of other phenomena from endomitosis to somatic reduction are explicable on the basis of timing differences between the two mechanisms.

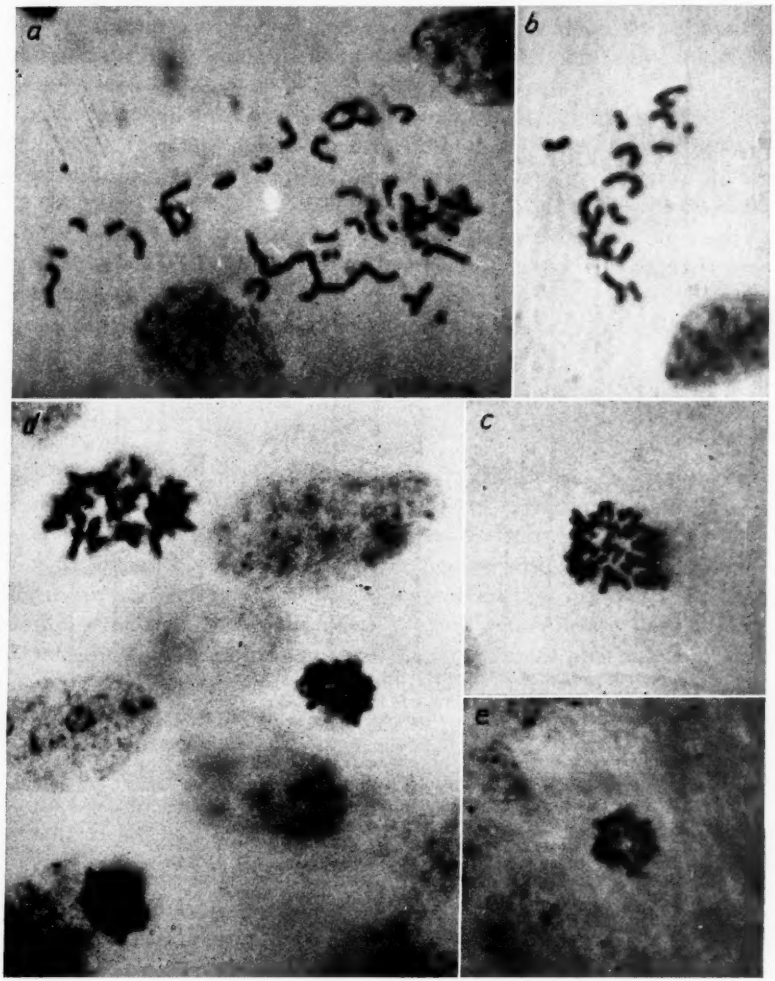

Fig. 3. *a*—*d*: mitotic stages from the uterine epithelium; *a*: prophase with 48 chromosomes; *b*: prophase with 16 chromosomes; *c*: metaphase with 40 chromosomes; *d*: metaphase with 44 chromosomes together with an anaphase in a lymphocyte with *ca.* 20 chromosomes; *e*: photo of the embryonic brain cell in Fig. 2 *c* with 27 chromosomes. (*e*: K, C, the others: AA, F.)

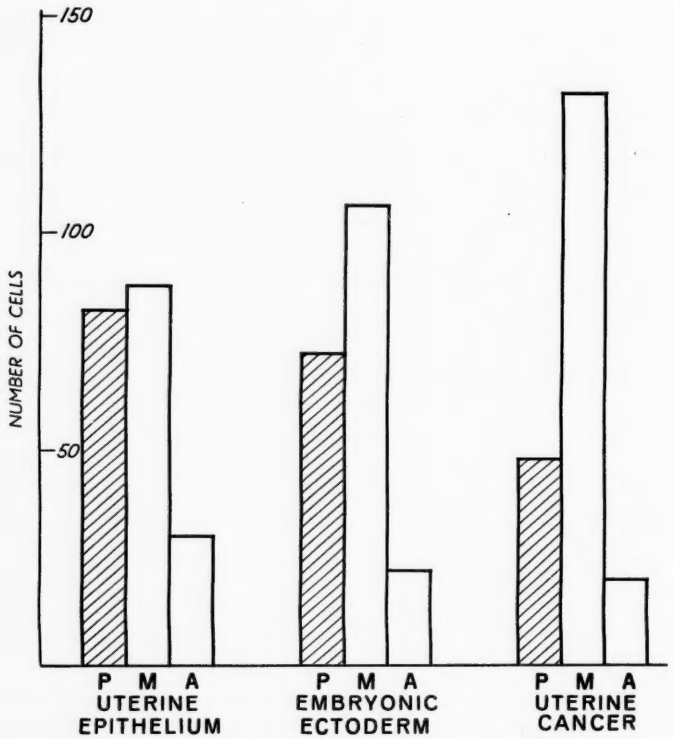

Fig. 4. The relative frequencies of prophase (P), metaphase (M), and anaphase (A), stages in uterine epithelium, embryonic ectoderm and uterine cancer as counted from 200 cells in each case.

According to the same line of thought, we have come to the conclusion (TIMONEN and THERMAN, 1950) that the original cytological change in cancer is that the extrachromosomal mechanism acts slightly in advance of the intrachromosomal mechanism. This is especially clearly seen in the relative shortening of the prophase stage, which is reflected by the decreased number of prophases as compared with metaphases.

In embryonic tissues also the relative duration of the prophase is shortened as compared with that of the adult uterine epithelium. Fig. 4 shows a count made of 200 dividing cells from normal uterine epithelium, embryonic ectoderm, and cancer tissue. It is seen that in

adult tissue the prophase is as long as, or longer than, the metaphase, in the embryonic tissue it is somewhat shortened, while in cancer it is greatly reduced when compared with the metaphase.

It has been proposed by us (TIMONEN and THERMAN, 1950) that the precocity of the spindle mechanism in cancer gives rise to the other irregularities observed. Similarly, the occurrence of laggards in embryonic cells might be explained as resulting from the slight precocity of the extrachromosomal mechanism in them. Fig. 2 *h* represents a typical anaphase from embryonic connective tissue with lagging chromosomes. As for other irregularities, it may be mentioned that multipolar divisions are not found, as a rule, in embryonic tissues, with but one exception in an embryonic liver cell.

IV. DISCUSSION.

Measurements of resting nuclei in various human tissues have shown that the nuclear volumes vary greatly, forming in many cases a geometrical series 1: 2: 4: 8, *etc.* (for references, see JACOBJ, 1935). This variation must obviously be connected with a similar variation in the chromosome number. The cells represent various levels of polyploidy, which obviously has resulted from endomitosis. This kind of somatic polyploidy is by no means an exceptional phenomenon, as is shown by a great number of observations on various organisms. It has been encountered both in plants and in many animals, especially in insects (*cf.* GEITLER, 1938; OKSALA, 1939).

The occurrence of polyploidy, however, differs in principle from the aneuploid variation in the chromosome number described in the present paper. It is probable that the uncertainty which for so long prevailed in regard to the chromosome number in man partly depended on the fact that the chromosome number actually varies in the different cells (for references, see ANDRES and JIV, 1936). It was only the examination of the germ line cells, especially in regard to spermatogenesis, which finally led to the conviction that the human chromosome number in both sexes was 48 (*cf.* MATTHEY, 1949).

When we examine the figures in the earlier papers representing human chromosomes, they appear technically so unsatisfactory that it is not surprising that evidence based upon them has been overlooked. Of the more reliable studies we may mention two. KARPLUS (1930) studied the foetal membranes and found variation in the chromosome number and complement of the cells. ANDRES and JIV (1936),

in a technically less satisfactory study, have determined the chromosome numbers in various embryonic tissues and found them to vary between 32 and 73. This evidence has not only, as a rule, been overlooked, but many cytologists, *e. g.* WINGE (1930) and especially KEMP (1930), have expressly stated all such data to depend solely on faulty observation.

Since every text-book of cytology contains the statement that every cell of a given organism contains the same constant chromosome complement, it is unnecessary to cite any particular paper on this point. Of those who have especially stressed the impossibility of aneuploid cells to live and function unassisted we may mention KOLLER (1947). Since it is well known that hypoploid cells occur in cancer tissues, KOLLER maintains that these are able to exist only when supported by cells with higher numbers of chromosomes. It was pointed out by us (TIMONEN and THERMAN, 1950) that whole cords of cancer tissue may consist of hypoploid cells. The present evidence makes it clear that hypoploid cells are able to function in normal human tissues also.

Several factors have certainly contributed to the neglect of evidence suggesting a variation in the human chromosome number. First, the germ line cells, especially in spermatogenesis, are the best available for investigation. From the chromosome number 48 found, it has not unnaturally been inferred that the cells of other tissues, too, contain the same number. This is the easier to understand as embryonic tissues especially are technically very difficult and thus have not inspired further studies. In the second place, plants and lower animals in which mitosis in somatic cells has been studied more extensively seem — except for occasional polyploidy — to have the exact diploid complement in their cells. Again the inference has been drawn that the same must be true of higher animals also. The third and certainly the most important point is, however, that such an aneuploid variation seems at first sight to be quite impossible to reconcile with the fact that heredity and gene action in man follow the same rules as in the lower organisms.

This fact is accounted for by us as follows. We must suppose that the original zygote giving rise to the organism contained 48 chromosomes. The embryo which has arisen from it has in its cells variable chromosome numbers and combinations. If we assume that every individual chromosome has divided the same number of times, the total number of every chromosome type in the organism will be the same. It seems that it does not affect the action of the chromosomes how they

are distributed between the cells. This might be expressed metaphorically as that the whole human organism acts as a giant polyploid cell in which the ratio of the different chromosome types has remained the same as in the original diploid complement.

For the present it is uncertain what the actual mechanism effecting the differential distribution of the chromosomes is. The only tissue in which chromosome counts have so far been made to a great extent is the uterine epithelium. TIMONEN's (1950) chromosome count of one thousand cells showed that the highest peak of frequency lies at 20—25 chromosomes and a much lower one at 45—50 chromosomes. In other words, the haploid and the diploid complement are most common. This may be interpreted as implying that these two combinations are most successful, *i. e.* cells containing them occur and/or divide most frequently. This is not surprising in view of the fact that in general balanced chromosome combinations function most satisfactorily. It must, however, be remembered that even cells in which the exact chromosome numbers 24 or 48 occur need not possess the total haploid or diploid complement, respectively, but they might contain different numbers and combinations of the various chromosome types.

The facts implied by the differential distribution of the chromosomes may be expressed as follows. The unit of organization in man is not the individual cell, but the entire organism acts as a single whole (*cf.* WIGGLESWORTH, 1948). A prerequisite for such a system to work similarly to an organism in which every cell contains a constant diploid chromosome complement is that the interaction between the cells should be much closer. In other words, the products of the genes, which in lower organisms are confined to a single cell, must be able to penetrate whole tissues. It may be pertinent in this connection that KEMP (1930), who maintains the human somatic chromosome number to be exactly 48, studied cells in tissue culture. It is possible that in tissue culture where each cell lives fairly independently in the same nutritive solution only the cells possessing the total diploid complement are able to survive.

A related phenomenon has been described by CLARK (1940) in an abnormal maize strain. In these plants the spindle in the first meiotic division is divergent, giving rise to several nuclei at each pole containing from one to several chromosomes. This leads to the formation of numerous small nuclei in the second division also. The interesting feature is that microspores with at least one complete set of chromosomes are able to develop independently of how many nuclei the chromosomes

are divided between. In the words of CLARK (*l. c.*, p. 556): »The chromosomal regulation of growth and differentiation of the microspore is not dependent upon the presence of the haploid complement within a *single* nucleus. The haploid chromosome complement in these cells can be separated by several nuclear membranes without disturbing the chromosomal control of normal developmental processes.» In this case the functional unit is the cell, as seems to be a characteristic feature in plants in general.

A fact which also lends support to our line of thought is that the impulses governing development and differentiation in man to a great extent act through the endocrine system. It is well known that the nuclei in gland cells form polyploid series. We may combine with this the hypothesis put forward by HUSKINS (1947, 1948) that gene action is closely correlated to or even dependent on the reproduction of genes. Then it does not seem improbable that gene action to a great extent takes place through the cells of the various glands, in which the volumes of the nuclei are increased through endomitotic reproduction of chromosomes. The chromosomes situated in other cells would affect development of the individual less, and thus a variation of the chromosome numbers between them would not have any harmful effects.

It is highly improbable that the system just described is confined to man. We therefore predict that it will be found in other animals, too. It is suggested tentatively that the boundary between this type of organization and that obviously prevailing in lower animals lies between warm-blooded and cold-blooded animals. On the one hand it is apparent that animals up to at least fishes and amphibians possess the diploid chromosome complement in their somatic cells. On the other hand very few studies have been made on the somatic chromosomes of warm-blooded animals. Carrying this idea further, it might be just the warm-blooded condition which makes such a system possible by intensifying metabolism. With this also accords the fact that in warm-blooded animals all physiological functions are more centralized and correlated than in lower animals.

Other evidence points in the same direction. Thus, the great capacity of regeneration characteristic of plants and lower animals might itself depend on the fact that every cell in them is a fairly independent genetical unit. The same explanation might apply to sex and other mosaics found, for instance, in insects and plants.

As pointed out above, our knowledge of the somatic chromosomes in higher animals is very deficient. The data described in the present

paper indicate strongly, however, that very interesting results could be obtained in this field. Doubtless they would throw quite new light on problems connected with development and differentiation. It may, in passing, be mentioned that the chromosomes of putative polyploids among higher animals ought to be investigated in greater detail before it can be decided with certainty whether they are actually polyploid. Should this be verified, another interesting question will be what effect the differential distribution of the somatic chromosomes will have on them.

We wish to acknowledge our gratitude to the Chief of the Women's Clinic, Professor A. TURUNEN, M. D., for excellent working facilities. For financial support we are indebted to Sigrid Juselius' Stiftelse.

SUMMARY.

(1) The human somatic chromosomes have been studied in the adult, the material used being the uterine epithelium in its proliferative stage, and various embryonic tissues: skin, brain, liver, connective tissue, intestine, *etc.* The chromosome number varied greatly in the cells, aneuploid numbers prevailing in all tissues.

(2) The chromosome number 48 is *not* the most common number outside the germ line. The highest peak of frequency, at least in the uterine epithelium, lies between 20 and 25 chromosomes, and there is a much lower one at 45—50 chromosomes.

(3) The question how the variation in the human somatic chromosome number originally arises is as yet open. Whether the occurrence of lagging chromosomes and other mitotic disturbances observed are able to bring about this variation is not clear on the present evidence.

(4) The variation in the somatic chromosome number may be reconciled with the Mendelian laws of heredity as follows. It is assumed that the 48 chromosomes present in the original zygote have, during the development of the embryo, divided the same number of times, although they are unequally distributed between the cells. A prerequisite for such a system to work similarly to that of the lower animals is that the physiological interaction between the cells is very close, *i. e.* that the gene-products penetrate the tissues easily. In addition it is probable that the endocrine glands are an important path through which the genes govern differentiation.

(5) We predict that the same type of aneuploid variation in the chromosome number will be found in other animals, too. The borderline between this type of organization and that characteristic of plants and lower animals lies most probably between warm-blooded and cold-blooded animals.

September 12th, 1950.

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INTER- AND INTRACLONAL CROSSES AND INBREEDING IN POTATOES

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SVALÖF, SWEDEN

INTRODUCTION.

THE potato varieties of the general market are all clones, selected among seedlings from crosses between older varieties. They are highly heterozygous, and it is rather probable that the breeder selects the most highly heterozygous seedlings, thus more or less consciously utilizing hybrid vigour. Studies of the effect of inbreeding in potatoes are mentioned by HAYES and IMMER (1942) without reference to authors. ASHTON (1946) mentions one American and one Russian study. KRANTZ and HUTCHINS (1929) have shown that selective inbreeding and crosses between inbred clones may result in high-yielding varieties. The inbred clones are on an average considerably poorer than their mother clones, but certain combinations of inbreds may give populations of seedlings superior to the initial material. GUERN (1940) has made a similar investigation. He also found a marked effect of inbreeding and that most combinations between inbred clones are poorer than the initial material, while quite a few may be superior.

The present study was not undertaken mainly in order to investigate the effect of inbreeding in potatoes but to check the correctness of the claims made by LYSENKO and his followers that crosses between plants within a clone — as between plants within a pure line — result in increased vigour, as compared to selfing. The results of LYSENKO are published in such a way as to make it impossible to check his statements, and new experiments may be desirable even if the existing material against his theories may well be considered as overwhelming.

A PRELIMINARY EXPERIMENT.

In 1945 crosses were made on a small scale between an old Swedish variety of unknown origin — »Old Red Swedish» — No. S 78 in the material of the Swedish Seed Association, and a clone of *andigenum*

type, H 132, from the Bolivian material of the Association. Besides the reciprocal interclonal crosses, pollinations were also made on a plant within the clone with pollen from other plants in the same clone, and, finally, artificial selfings were carried out.

Most of the flowers pollinated set fruits, except the selfed ones of S 78, and the number of seeds per fruit was fairly high, as seen in column 5, Table 1.

TABLE 1. *Data from a preliminary experiment in 1945—46.*

1	2	3	4	5	6	7	8	9	10	11	12	13
Field No.	Combination	Number of flowers pollin.	Number of fruits obt.	Seeds per fruit	1000 seeds, grams	Seeds sown 20/3	Number of seedlings 29/3	2/4	3/4	6/4	%	Plants in field in % of seeds
043	H 132 × S 78	7	7	262	0,58	500	102	373	437	440	88	71
044	H 132, selfed	5	5	144	0,51	500	2	133	243	248	50	35
045	H 132, between plants	5	5	230	0,62	500	4	149	328	329	66	25
046	S 78 × H 132	8	7	117	0,56	578	13	303	434	436	75	61
047	S 78, between plants	10	3	57	0,52	171	2	70	97	105	61	39

The weight of seeds was higher after intraclonal crossing than after selfing in H 132 (col. 6). The number of seeds sown in the spring of 1946 and the number of seedlings sprouted at different intervals are shown in columns 7—12. The germination percentage as well as the germination energy (measured by the number of days until sprouting) is markedly lower after selfing and intraclonal crossing than in the interclonal crosses. Again, the seeds after the intraclonal cross in H 132 seem to be more vigorous than those after selfing. A difference in vitality between the reciprocal crosses, 043 and 046, is indicated. The seedlings were transplanted in cold benches and later on to the field, a number of them dying in these processes. Column 13 gives the number of surviving seedlings in percentage of seeds sown. Here, again, the seedlings after selfing are weakest, but these figures do not indicate an increase in vigour after intraclonal crossing.

This preliminary experiment showed an evident effect of inbreeding, and also gave a certain indication that intraclonal crossing might increase the vigour in comparison with strict selfing. The material was small, however, and, furthermore, the clone H 132 was fairly old. It was also conceivable that mutations might have occurred and caused

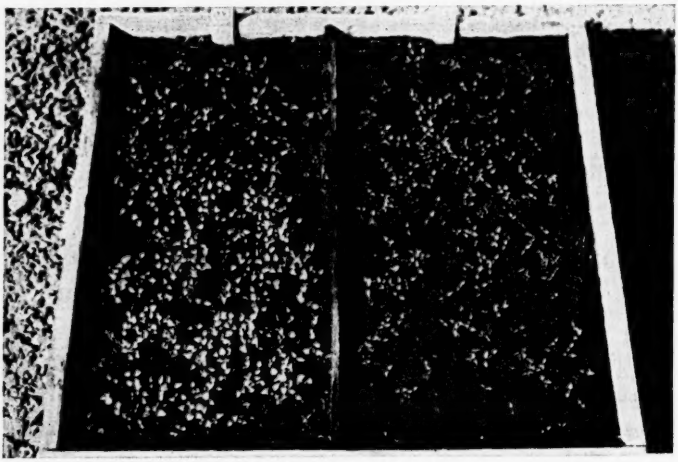

Fig. 1. Potato seedlings after interclonal crossing (043; to the left) and after selfing (044; to the right). For numbers, see Table 1. Photographed on April 3rd, 1946.

some genetical variation within the clone. The experiment was repeated and a new and more comprehensive experiment was started, using quite young clones.

The crosses including H 132 and S 78 were repeated in 1947, the seed sown in 1948 and the main results being summarized in Table 2.

TABLE 2. *Results from a second experiment in 1947—48.*

Combination	Fruits in % of flowers pollinated	Seeds per fruit	Seedlings in % of seeds sown
H 132 × S 78	100	238	83
H 132, selfed	64	40	71
H 132, between plants	46	42	74
S 78 × H 132	83	162	73
S 78, selfed	57	57	75
S 78, between plants	42	62	76

The results confirm the effect of inbreeding and also indicate a difference between reciprocal crosses, but in this material there is no indication whatever of an effect of interclonal crossing. The question is still open whether the indication of such an effect obtained in the

first experiment was a purely random occurrence, or if the intraclonal cross in 1945 happened to be made between two genetically different plants within the clone. The plants were, unfortunately, not separately harvested, and the experiment could not be exactly replicated. We have not carried this part of the study further, since the results to be reported in the next section seem to be conclusive as regards the main issue.

RESULTS OF CROSSES MADE WITH YOUNG CLONES.

In 1946 and 1947 a series of crosses, including different types of combinations, were made. The material used comprized plants from the first clonal generation of new seedlings grown in 1945 and 1946, respectively, richly flowering clones being used. The clones were combined two and two, and one plant in each was used as mother plant for all combinations. All plants used were harvested separately and their clonal offspring grown the next year, so as to offer possibilities for exact replications of the combinations if desirable. The combinations made and the symbols used may be exemplified by the series of crosses in the clone-pair 701 and 703.

701 plant 1 × 703 plant 1, interclonal cross, symbol Cr_1
701 » 1 × 701 » 1, selfing, symbol S_1
701 » 1 × 701 » 2, intraclonal cross, symbol 2_1
701 » 1 × 701 » 3, intraclonal cross, symbol 3_1
703 » 1 × 701 » 1, interclonal cross, symbol Cr_2
703 » 1 × 703 » 1, selfing, symbol S_2
703 » 1 × 703 » 2, intraclonal cross, symbol 2_2
703 » 1 × 703 » 3, intraclonal cross, symbol 3_2

As a rule, 5—10 flowers were pollinated in each combination. In each of the two years three complete series were obtained, in the other pairs one or more of the combinations were unsuccessful. The seeds from these six series were sown in the springs of 1947 and 1948, respectively. Table 3 gives the data from these series, all the combinations of the same type in each year being pooled.

These data again confirm the effect of inbreeding, but there is no consistent difference between strict selfing and intraclonal crossing. In 1947 the seedlings were reasonably well developed in the field; in

TABLE 3. *Results from inter- and intraclonal crosses and selfings in 1946 and 1947.*

Type of combination and year	Fruits in % of poll. flowers	Seeds per fruit	Weight per fruit, grams	Seedlings in % of seeds sown			Plants in the field in percentage of seeds sown			Tuber weight per plant, kilo
							planted on	surviving on	harvested on	
1946—47				28/4	2/5	5/5	18/6	18/7	8/10	
Cr	27,2	101	3,42	31	58	65	26	26	24	0,24
S	27,1	90	3,46	24	52	60	22	21	20	0,14
2 + 3	26,9	95	3,07	26	52	59	23	21	17	0,15
1947—48				30/3	7/4	12/4	14/6	27/7	29/9	
Cr	26,8	156	4,24	49	83	89	21	15	14	0,17
S	28,1	127	4,29	19	78	81	14	8	7	0,12
2 + 3	28,3	111	3,76	32	76	84	18	11	9	0,12

1948 the seedling cultures were very poorly developed. Only the field material from 1947 (crosses 1946) has therefore been used for a detailed analysis of the tuber yield of the seedlings from different combinations.

The clones used in this material and their origin are as follows:

Pair A: a) 46/796 = seedling from the cross Ostbote × Pepo.
b) 46/839 = seedling from the cross Voran × (King George V × H 28).

(H 28 is a clone of South American origin.)

Pair B: a) 46/1051 = seedling from the cross Ackersegen × Sandnudel.
b) 46/1079 = seedling from the cross Ackersegen × Stärkereiche I.

Pair C: a) 46/1132 = seedling from the cross Voran × Konsuragis.
b) 46/1218 = seedling from the same cross as A [b)].

The tuber yield of each seedling in all combinations in these three series has been weighed, and the data have been subjected to an analysis of variance. Table 4 gives the number of seedlings and the average tuber weight per seedling in kilos in all combinations.

TABLE 4. *Number of seedlings and average tuber weight per seedling in kilos in material grown in the field in 1947.* See text.

Combination	Pair A		Pair B		Pair C	
	Number of seedlings	Weight per seedl.	Number of seedlings	Weight per seedl.	Number of seedlings	Weight per seedl.
a) × b), interclonal	48	0,31	44	0,28	54	0,27
a) × a), selfing	29	0,19	24	0,17	21	0,17
a) × a), intraclonal, 2	29	0,14	22	0,21	13	0,11
a) × a), » , 3	19	0,15	27	0,21	14	0,19
b) × a), interclonal	60	0,25	21	0,18	36	0,14
b) × b), selfing	26	0,15	12	0,15	5	0,04
b) × b), intraclonal, 2	11	0,16	10	0,09	19	0,16
b) × b), » , 3	14	0,19	17	0,12	5	0,14

The analyses of variance are summarized in Table 5. The numbers of degrees of freedom are different in the different pairs, being 235, 176 and 166 for total variation and 7 less for error in pairs A, B and C, respectively.

TABLE 5. *Analyses of variance of the data summarized in Table 4.*

Cause of variation	De-grees of free-dom	Pair A		Pair B		Pair C	
		Vari-ance	Quotient	Vari-ance	Quotient	Vari-ance	Quotient
Total		0,0143	—	0,0220	—	0,0232	—
Between combinations	7	0,1290	$11,94^{***}$	0,0773	$3,92^{**}$	0,1177	$6,16^{***}$
Interclonal: all others	1	0,7397	$68,49^{***}$	0,2285	$12,22^{***}$	0,2916	$15,27^{***}$
Reciprocal interclonal	1	0,1046	$9,69^{**}$	0,1319	$6,70^{*}$	0,3856	$20,19^{***}$
all others, a) : b)	1	0,0010	1	0,1339	$6,80^{**}$	0,0773	$4,05^{*}$
selfing: intraclonal, a)	1	0,0424	$3,93^{*}$	0,0283	2	0,0035	1
» : » , b)	1	0,0089	1	0,0126	1	0,0189	1
intraclonal 2:3, a)	1	0,0018	1	0,0006	1	0,0383	2,01
» » , b)	1	0,0045	1	0,0056	1	0,0084	1
Error		0,0108	—	0,0197	—	0,0191	—

We have used the method suggested by BONNIER and TEDIN (1940) to denote significance; *** = P 0,001, ** = P between 0,01 and 0,001, * = P between 0,05 and 0,01.

The difference between interclonal crosses and other combinations was evident during the summer season, the top being much weaker in the latter group. The analysis of variance confirms the difference

as regards tuber yield. In none of the three pairs was there any indication of a stimulating effect of crossing two plants within a clone; in the only instance where the difference between selfing and intraclonal crossing reaches any level of significance the progeny after selfing has the higher yield. The data from crosses made in 1947 with seedlings grown in 1948, although not given here in detail, confirm the results.

There is a marked and more or less significant difference between the reciprocal combinations of interclonal crosses in all three pairs, a fact which will be discussed in the next section.

DISCUSSION.

That selfing of the potato results in inbreeding degeneration was evident in this experiment as in others previously made by other authors. There is no proof whatever of a stimulating effect of crossing different plants within a truly isogenic clone. Some indications were obtained in a preliminary experiment, but they were open to criticism and, when the experiment was repeated on a sufficiently large scale with a reasonable guarantee for the genotypical uniformity of the clone, the intraclonal crosses gave the same degree of inbreeding degeneration as the true selfings.

There is a marked difference between the reciprocal interclonal crosses. These differences cannot be due to environment in the field, since the plots were so distributed as to eliminate soil differences to a large extent, and since the phenomenon occurs in three different pairs in 1947 and also recurred in 1948. The explanation is perhaps to be found in the differences between the fertility in both sexes. As is well known, the male fertility of the potato is often more or less reduced. The female fertility (as measured by the number of seeds per fruit) is only weakly (statistically insignificant in this material) or not at all correlated to the male fertility (as measured by the quality of the pollen) of the same clone, a fact corroborated by other data (HAGBERG, unpublished). The number of fruits obtained in percentage of flowers pollinated is, further, weakly or not at all correlated to the male fertility of the mother clone. The elimination of gametes seems to be much stronger on the male side, and it seems to be a reasonable assumption that this elimination is selective, so that the »gene-population» functioning in the male sex will be different from the one functioning in the female. In this connection it should be pointed out, however, that in

the pairs B and C there is a more or less significant difference between the inbred progenies of the two clones of the pair. In both cases the clone giving the lowest yield after selfing is also the one which gives the lowest yield when functioning as mother-plant in the interclonal cross. This fact indicates that the causation of the reciprocal differences may be more complicated than the one just suggested.

Lastly, it may be pointed out that the seed setting is much better after crossing than after selfing. This is in no way astonishing, since the potato must be regarded as a typical cross-fertilizer, in spite of the fact that all clones tested by us have a higher or lower degree of self-fertility, and many commercial varieties a very high one, indeed.

SUMMARY.

(1) There is a marked degeneration in the F_1 after inbreeding.

(2) No stimulating effect of crossing different plants within a clone has been obtained.

(3) There are marked differences between the progenies of reciprocal interclonal crosses. As an explanation is suggested differential elimination of genes in both sexes, but the phenomenon may very well be more complicated.

(4) The seed setting is better after interclonal crossing than after selfing within the clone.

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ABSTRACTS

YNGVE MELANDER: Polyploidy after colchicine treatment of pigs.

According to KRALLINGER (1931), BRYDEN (1933), and MULDAL (1948), the domestic pig has $2n = 38$. Seven specimens from a large pure breed of Yorkshire pigs (at BONDESSON's, Svalöf) which I have studied also have 38 mitotic chromosomes and 19 bivalents in testicular tissue.

However, animals from three breeds of »Old Swedish» pigs turned out to have only 30 chromosomes instead of 38 in their testicles, whereas on a farm where both races had previously been crossed the chromosome number characteristic of each pig varied within the breed from 30 to 36 in the animals studied.

Before the chromosome conditions had become known HÄGGQVIST and BANE started experiments, on the last-mentioned farm, with artificial insemination of sperm mixed with colchicine, their object being to produce triploid pigs (see HÄGGQVIST and BANE, 1950). The experiments were carried out on three sows with sperm from one boar.

Among the resulting 31 young pigs one male deviated by having markedly larger erythrocytes at birth (communicated by HÄGGQVIST). Later, one of the testicles was removed from this particular animal and from his brothers and half-brothers. His mitotic chromosome number in the testicle is most probably 47. It is quite certainly over 45 and not more than 48. The brothers and half-brothers had chromosome numbers from 30 to 34.

This can be explained on the supposition that the father, which is of »Old Swedish» race, produces spermatozoa with 15 chromosomes and the mothers, which are probably of mixed race, egg-cells containing 15 to 19 chromosomes. Thus, the number 47 will result after the union of an egg-cell — colchicine doubled at the second meiotic division and having $2 \times 16 = 32$ chromosomes — with a sperm having 15 chromosomes.

All pigs studied have only one characteristic chromosome number in the testicles. Aneuploid cells are not very common. This is also the case with the animal supplied with 47 chromosomes. There is no variation like that observed by the author in a colchicine treated rabbit [MELANDER, 1950; it was supposed at the time that multipolar spindles might have caused the formation of different chromosome numbers in that rabbit, a supposition later confirmed on another young rabbit, which originated in HÄGGQVIST and BANE's experiments; about 1 % of its mitotic divisions in the testicles were tetrapolar, having a chromosome number estimated at about 66, whereas the number 33 prevailed in mitotic plates of both red bone-marrow and testicles]. Nor is there any variation like that found by THERMAN and TIMONEN (1950) in different tissues of man.

The fixative was 1—3 acetic alcohol and the chromosome preparations were permanent FEULGEN squashes.

The author has started a close study of the chromosome conditions of pigs including crosses.

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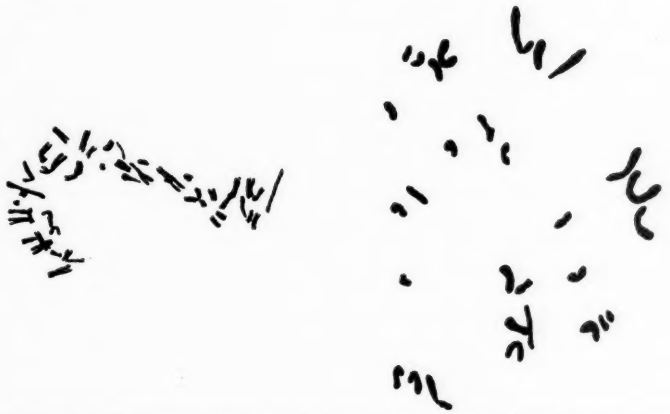

Fig. 1. Mitotic chromosome plates of two pigs. One has 47 minute chromosomes as a result of colchicine treatment. The other (normal) animal has 30 larger chromosomes.

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IRMA ANDERSSON-KOTTÖ: Mutations in antiserum treated *Neurospora*.

Induction of mutations by antibodies in *Neurospora* was reported by S. EMERSON (1944). The mutations differed from the parental types in morphological characters or by deficient growth. Pending a more thorough investigation of this or more favourable material some experiments with *Neurospora* by the present author may contribute some facts. Macroconidia from the wild type strains 25 a and 1 A, the strains kindly provided by Professor G. W. BEADLE, and No. 260, a descendant from 25 a, as well as microconidia from a macroconidieless strain No. 237, were treated with antiserum and families raised directly from isolated conidia without fertilization. Only biochemical mutations are recorded.

Material treated	Treatment	Time	Temperature	Total isolates normals	mutants
Macrocon. of 25 a	100 % antiserum	24 hrs.	2°	87	0
»	»	»	23°	92	1
»	—	»	»	106	0
»	normal serum	»	»	226	0
Macrocon. of 1 A	100 % antiserum	»	2°	101	0
»	»	12	23°	77	2
»	—	»	2°	116	0
Macrocon. of 260	100 % antiserum	1—24	2 and 23°	350	0
Microcon. of 237	»	»	»	606	0
»	—	»	»	187	0

Whether the appearance of the mutations is caused by direct induction or by selection they seem to appear in these experiments at 23° from macroconidia in the wild type strains, none occurring either from macro- or microconidia in the other two strains or in the controls.

No differences were found in the antigenic characters, as determined by complement fixation tests, in wild type strains of *Neurospora crassa. tetrasperma* and *sitophila*, their normal descendants and several biochemical mutants; neither have mutations in the antigenic character appeared after treatment with antiserum, X-ray or mustard gas.

The work was undertaken with grants from Wenner-Grenska Samfundet and Statens Naturvetenskapliga Forskningsråd.

Stockholm, Wenner-Grens Institut.

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ANTERO VAARAMA: Chromosome number and cryptic polyploidy in *Lepidium sativum*.

Knowledge of the chromosome number of the common cress, *Lepidium sativum*, is based primarily on the investigation of JARETZKY (1929). He reports, without giving any hint of the source of his material, the haploid chromosome number $n = 8$. Though there are, in fact, types with $2n = 16$ chromosomes among the cultivated forms, as can be seen from the recent paper of REESE (1950), the present author has not been able to verify this chromosome number. On the contrary, all the material studied has shown without exception the somatic chromosome number $2n = 24$. The origin of the material investigated is as follows:

(1) Several samples of commercial seed originating from different Finnish seed firms.

(2) A sample of seed from the Agricultural Experimental Station, Sofia, Bulgaria.

(3) Several samples of seed from the Institute of Crop Plant Research, Gatersleben, Germany. This material includes the following taxonomical units:

Lepidium sativum var. *vulgare* subvar. *typicum* THELL., »common green»

Lepidium sativum var. *vulgare* subvar. *crispum* (MED.) DC., »extra crinkled»

Lepidium sativum var. *vulgare* subvar. *crispum*, »extra crinkled green»

Lepidium sativum var. *vulgare* subvar. *latifolium* DC.

The chromosome number of a total of 11 seed samples has been determined. The two last-mentioned forms from Gatersleben originate from N. W. India, having been brought from there by the German Hindukush expedition. This fact reveals that the chromosome number $2n = 24$ also occurs in the region which has been considered as the centre of origin of this crop plant (*cf.*, *e. g.*, GRAM, JENSEN and MENTZ, 1937; DARLINGTON and JANAKI AMMAL, 1945).

Unfortunately, my material does not contain the wild type of *Lepidium*, *viz. L. sativum* var. *silvestre* THELL., considered as the ancestral form of the cultivated common cress. The considerably smaller size of its seeds is, however, a strong indication of the existence of a chromosome number lower than $2n = 24$. Neither has the chromosome number of the form of *L. sativum* growing as a weed of flax been studied. Morphologically, it has been considered to be at least very near the cultivated form, and we should thus expect the same chromosome number. A cytological examination of the wild forms would be necessary to elucidate the evolutionary history of the cultivated *L. sativum*, which seems to be a matter of controversy (*cf.* BERTSCH, 1947; HJELMQVIST, 1950).

MANTON (1932), in her study on the chromosome numbers in the family Cruciferae, has given a list of the chromosome numbers in the genus *Lepidium*. There we can see a polyploid series with the basic chromosome number $x = 8$. We might thus consider *L. sativum* as a triploid species. In its fertility and meiosis we cannot, however, find any signs of triploidy. Meiosis has been extremely regular, pollen grains good and seed formation abundant. We cannot, therefore, maintain the idea that *L. sativum* with the chromosome number $2n = 24$ is really triploid. Indeed, we can hardly imagine a mechanism which could establish triploidy, especially in an annual plant species propagating only by seed.

That *L. sativum* might be a hexaploid species with a basic chromosome number $x = 4$ would seem to be better in accordance with the facts observed. This basic number is not known in the family Cruciferae (MANTON, *l. c.*). We have, however, reason to suppose that this ancient basic number is hidden, at least in the genus *Lepidium*, in all the chromosome numbers divisible by four. The case is analogous with *Ribes nigrum*, in which the present author has been able to demonstrate a similar case of cryptic polyploidy in the »diploidized» chromosome number $2n = 16$ (VAARAMA, 1949).

There is also another species in the genus *Lepidium*, *viz. L. latifolium* L., which according to MANTON (*l. c.*) (»probably») has a chromosome number $2n = 24$. It is, like *L. sativum*, an annual plant propagating by seed. The

chromosome number of the form *L. cartilagineum* var. *crassifolium* W. K., $2n = 40$ (MANTON, *l. c.*), would also be easier to fit in with the other chromosome numbers established in the genus by supposing the basic chromosome number $x = 4$ as suggested above.

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